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CONTENTS

Introductory Remarks. *By* D. Wayne Woolley 553
Amino Acid and Fatty Acid Hydrazides: Chemistry and Action on Monoamine Oxidase. *By* P. Zeller, A. Pletscher, K. F. Gey, H. Gutmann, B. Hegedüs, and O. Straub 555
Structure and Activity Relationships of Monoamine Oxidase Inhibitors. *By* John H. Biel, Patrick A. Nuhfer, and Alvin C. Conway 568
In Vitro and *in Vivo* Inhibition of Amine Oxidases. *By* E. A. Zeller, L. A. Blanksma, W. P. Burkard, W. L. Pacha, and J. C. Lazanas 583
Pharmacology of JB-516 (PIH). *By* Akira Horita 590
Biochemical and Pharmacological Studies of β-Phenylethylhydrazine and Selected Related Compounds. *By* Max Chessin, Bernard Dubnick, Gerald Leeson, and Charles C. Scott 597
Interaction of Monoamine Oxidase Inhibitors with Physiological and Biochemical Mechanisms in Brain. *By* Bernard B. Brodie, Sydney Spector, and Parkhurst A. Shore 609
Effects of Amine Oxidase Inhibitors on Cerebral Cortical Responses to Epinephrine. *By* Bruno Minz and E. J. Walaszek 617
Pharmacology of Iproniazid and Other Amine Oxidase Inhibitors. *By* Lowell O. Randall and Robert E. Bagdon 626
Anticonvulsant Properties of Monoamine Oxidase Inhibitors. *By* Darwin J. Prockop, Parkhurst A. Shore, and Bernard B. Brodie 643
Comparison of Iproniazid with Other Amine Oxidase Inhibitors, Including W-1544, JB-516, RO 4-1018, and RO 5-0700. *By* Samuel d'A. Bailey, Luigi Bucci, Ernest Gosline, Nathan S. Kline, In Ho Park, Dina Rochlin, John C. Saunders, and Maurice Vaisberg 652
The Inert Psychasthenic Reaction (Anhedonia) as Differentiated from Classic Depression and Its Response to Iproniazid. *By* Leo Alexander and Austin W. Berkeley .. 669

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Clinical Experiences with Amine Oxidase Inhibitors in Psychiatry. *By* ARNO VOELKEL . . 680
Clinical Experience with Newer Antidepressant Drugs and Some Related Electroencephalographic Observations. *By* DOUGLAS GOLDMAN 687
The Use of JB-516 in Psychiatry. *By* HENRY V. AGIN 705
Treatment of Hospitalized Mental Patients with Iproniazid. *By* PAUL E. FELDMAN . . . 712
The Influence of Amine Oxidase Inhibitors on Epinephrine Metabolism in Man. *By* OSCAR RESNICK 726
Chemical Treatment of Depression. *By* FRANK J. AYD, JR. 734
The Use of Iproniazid in the Treatment of Depressive States. *By* J. ROBERT HAWKINS, WILLIAM E. HILLARD, JAMES A. L. MOULTON, AND HOWARD FABING 745
The Usefulness of Amine Oxidase Inhibitors in Psychiatry. *By* SHERVERT H. FRAZIER AND ROBERT L. FAUCETT 754
Clinical Evaluation of Psychostimulants. *By* JOSEPH M. TOBIN, MARC A. PLANTE, AND LESTER KUPSKI 760
Relationship of Iproniazid to Epinephrine Metabolism. *By* A. HOFFER 772
The Phrenopraxic Activity of a Nonnoxious Antidepressant. *By* ANTHONY SAINZ 780
The Place of Iproniazid in Biochemical Psychiatry. *By* WILSON G. SCANLON 797
Iproniazid: A Brief Review of Its Introduction and Clinical Use. *By* DAVID M. BOSWORTH 809
The Effects of Iproniazid and Other Amine Oxidase Inhibitors in Rheumatoid Arthritis. *By* ARTHUR L. SCHERBEL AND JOHN W. HARRISON 820
Experience with JB-516 and Other Psychochemicals. *By* JULIUS POMERANZE 835
The Use of JB-516 in Neuromuscular Conditions in Pediatrics. *By* M. A. PERLSTEIN . . 843
Effects of Iproniazid and Similar Compounds on the Gastrointestinal Tract. *By* JEROME WEISS, SAMUEL WEISS, AND BERNARD WEISS 854
Some Observations on the Use of Iproniazid in the Management of Craniocerebral Trauma. *By* GERALD R. NOWLIS 860
The Metabolism of Iproniazid *By* B. KOECHLIN AND V. ILIEV 864
Experimental Pathology of Iproniazid and Related Compounds. *By* GERHARD ZBINDEN AND ALFRED STUDER 873
The Relationship of Certain Vitamin Deficiencies to the Toxicity of Iproniazid. *By* FRED ROSEN 885
Serial Hepatic Study in Patients Treated with Iproniazid. *By* RICARDO KATZ, JAIME KLINGER, LUIS SILVA, JULIO RODRIGUEZ, AND HECTOR DUCCI 898
Some Preliminary Clinical and Biochemical Aspects of Liver Function in Patients Receiving Iproniazid. *By* H. J. ZIMMERMAN, L. ROSENBLUM, R. J. KORN, AND P. E. FELDMAN 915
Hepatic Injury in Patients Who Have Received Iproniazid. *By* HANS POPPER 928
Changes in the Intrahepatic Circulation and the Reticuloendothelial System Induced by Inhibitors of Monoamine Oxidase. *By* BENJAMIN W. ZWEIFACH AND JOSEPH CASCARANO 939
Clinical Toxicity Studies of a Monoamine Oxidase Inhibitor. *By* I. J. GREENBLATT AND A. KAHN 947
Serotonin and the Dynamics of the Heart. *By* C. W. CRUMPTON, C. A. CASTILLO, G. G. ROWE, AND G. M. MAXWELL 960
A Method for Measurement of Monoamine Oxidase Inhibition in Man: Application to Studies on Hypertension. *By* ALBERT SJOERDSMA, LOUIS GILLESPIE, AND SIDNEY UDENFRIEND 969
Effect of Iproniazid on Survival in the Dog, Following Acute Coronary Occlusion. *By* W. REGELSON, F. S. HOFFMEISTER, AND H. WILKENS 981
Iproniazid in Cardiovascular Therapy, with Special Reference to Its Action in Angina Pectoris and Blood Pressure. *By* TEODORO CESARMAN 988
Contribution of Iproniazid to the Treatment of Angina Pectoris. *By* PEDRO COSSIO . . . 1009
Isopropylhydrazides in Angina Pectoris. *By* WALTER SCHWEIZER 1016
Iproniazid in the Anginal Syndrome Due to Coronary Disease. *By* ARTHUR M. MASTER AND EPHRAIM DONOSO 1020
Significance of Monoamine Oxidase Inhibition for the Pharmacological and Clinical Effects of Hydrazine Derivatives. *By* A. PLETSCHER 1039

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INTRODUCTORY REMARKS

D. Wayne Woolley
The Rockefeller Institute, New York, N. Y.

There is a group of hormones, each one of which causes smooth muscles to contract; similarly, each one of these hormones has been implicated in the normal functioning of the nervous system. These hormones are serotonin, adrenaline, noradrenaline, acetylcholine, and histamine. There are probably others as well that are still unknown. We are concerned with some of these hormones, namely, serotonin, adrenaline, and noradrenaline, in this monograph which discusses a group of drugs that cause increases in the concentrations of them in the body.

First, let us consider some basic physiology that recently has been found to apply to each of these hormones. The hormone is formed by a specific enzymic reaction. It then seems to be stored away in some cellular particle, in which form it is pharmacologically inactive. For serotonin, one such particle is the platelet of the blood. When the hormone is released from this particle it becomes pharmacologically active and able to cause muscular contraction or nerve activity. Once the hormone has acted upon its target cell, it must be disposed of in order to avoid a continuing effect that might be physiologically undesirable. For this purpose a destructive enzyme is provided. It is probably for this reason that one finds monoamine oxidase in smooth muscles and in nerves. This enzyme actively destroys serotonin, noradrenaline, and adrenaline, which are monoamines. We may view choline esterase, which rapidly destroys acetylcholine, in a similar light. The result of inhibiting such a destructive enzyme is to allow accumulation and continuing action of the hormone on muscles and on nerves. The inhibition of an enzyme of this sort is thus one way of increasing the physiologically acting hormone in the body. Another publication of The New York Academy of Sciences treats of the pharmacological effects and therapeutic usefulness of drugs that inhibit choline esterase and thereby increase the amount of acetylcholine in the body.[1] In the present publication we consider similar events that occur when drugs are used that inhibit amine oxidase and thereby increase the concentrations of serotonin, noradrenaline, and adrenaline in the body.

The reader may wonder why one would wish to increase the amount of serotonin and the adrenalines. I believe that the attempt to do this for serotonin arose largely from the suggestion of Woolley and Shaw, published early in 1954.[2] These investigators produced considerable evidence to suggest that some mental disorders may arise from abnormalities in, probably a deficiency of, the serotonin content of the brain. They further suggested that perhaps a rational way to attempt treatment of these disorders would be to increase the serotonin content of the brain in order to make up the deficiency. There are several different ways in which this might be done. One would be to increase the production of the hormone; this is the method that we have been exploring in our own laboratory for the past several years. Another way would be to inhibit destruction of the hormone. The results of attempts to do this constitute the subject of the present publication, as the amine oxidase inhibitors have been used principally for this purpose.

I view with much satisfaction that success in the treatment of certain mental disorders that has attended the use of these inhibitors. This success tends to vindicate our conclusions as to the nature and treatment of certain mental diseases.

The implication of noradrenaline in mental disorders has recently come to the fore both from the findings of its role in the transmission of nerve impulses and its occurrence in the hypothalamus, as well as from the observation that a tranquilizing drug, namely, reserpine, displaces this hormone from the brain, especially from the hypothalamus. Holzbauer and Vogt, in 1956,[3] were the first to observe this, and they were followed by many others. For example, Brodie and his associates have studied this phenomenon both for noradrenaline and earlier for serotonin.[4] Much evidence suggests that both noradrenaline and serotonin may be of importance in the functioning of the brain as reflected by psychiatric change. There is a long-standing belief that adrenaline may also be concerned. Drugs such as the amine oxidase inhibitors, which increase each of these hormones in the body, are consequently of much interest.

Aside from these rational approaches to the problem, chance has played a role in the introduction into medicine of amine oxidase inhibitors. I understand that the chance observation that iproniazid caused euphoria in tuberculous patients was one of the reasons for the testing of this drug on depressed mental patients. Because these observations made by chance were contemporary with the discoveries that suggested that certain mental diseases might be treated usefully by increasing brain serotonin, it is almost impossible to say how the motivation was divided.

References

1. ANNALS OF THE NEW YORK ACADEMY OF SCIENCES. 1957. Curare and Anticurare Agents. **54**(3): 297–530.
2. WOOLLEY, D. W. & E. SHAW. 1954. Proc. Natl. Acad. Sci. U.S. **40:** 228.
3. HOLZBAUER, M. & M. VOGT. 1956. J. Neurochem. **1:** 8–11.
4. BRODIE, B. B., J. S. OLIN, R. G. KUNTZMAN & P. A. SHORE. 1957. Science. **125:** 1293–1294.

AMINO ACID AND FATTY ACID HYDRAZIDES: CHEMISTRY AND ACTION ON MONOAMINE OXIDASE

P. Zeller, A. Pletscher, K. F. Gey, H. Gutmann,
B. Hegedüs, O. Straub
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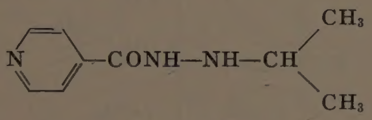
Iproniazid (Marsilid*) interferes with different enzyme systems. Evidence exists that monoamine oxidase (MAO) inhibition is responsible for some of the actions of the drug in humans, such as the beneficial effects in mental depression and angina pectoris. On the other hand, the many side effects may also be due to this inhibition, which takes place in a variety of organ systems. It was believed that, by increasing the organ specificity of the molecule, side effects could be diminished or avoided.



Iproniazid

Therefore, modification of the alkyl as well as the acyl part of the molecule was carried out as the first step of a research program. This showed that the inhibitory effect on MAO of hydrazides depends on both the acyl and the alkyl parts of the molecule. The hypothesis was put forward that, by selection of the proper acyl radical, the organ specificity of the hydrazides could be improved. Thus, natural amino acids with a high turnover in a particular organ might carry the active hydrazine molecule to the organ (heart or brain) where action is desired and keep it away from the organ (liver) where it might cause side effects.

Evaluation of the potency of the compounds was done by measuring the rise of 5-hydroxytryptamine (5-HT) in rat brain produced by an amount equimolar to 100 mg./kg. iproniazid 16 hours after application. In the following discussions the relative increase of 5-HT as compared to iproniazid will be called the Marsilid index, defined as follows:

$$\text{Marsilid index} = \frac{\textit{Increase of 5-HT in rat brain produced by a substance in amount equimolar to 100 mg./kg. iproniazid}}{\textit{Increase of 5-HT in rat brain produced by 100 mg./kg. iproniazid}}\ 100$$

Table 1 shows many of the compounds tested that are derived from heterocyclic or aromatic acids. In order to study the effect of variation in the acyl residue, the remainder of the iproniazid molecule (isopropylhydrazine) was left unchanged. No generalization concerning molecular structure and activity could be deduced from the results obtained.

Table 2 shows some of the compounds having an amino or aliphatic acid as

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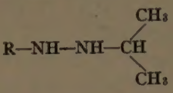
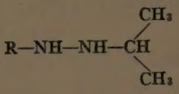
* Hoffmann-La Roche.

TABLE 1

Heterocyclic acids		Aromatic acids	
 $R{-}NH{-}NH{-}CH(CH_3)_2$		 $R{-}NH{-}NH{-}CH(CH_3)_2$	
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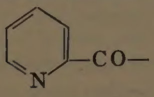
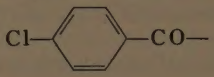
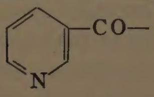
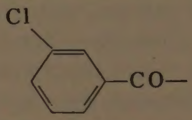
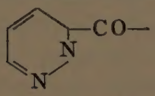
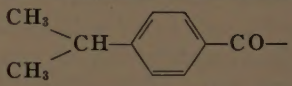
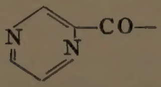
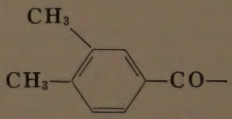
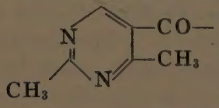
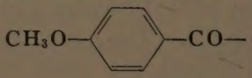
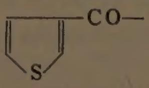
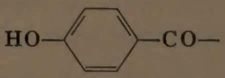
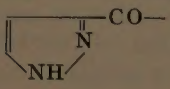
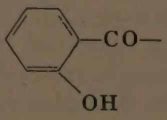
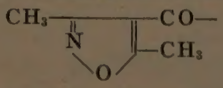
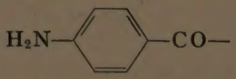
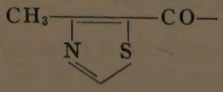
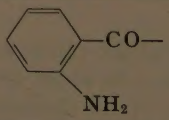
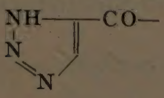
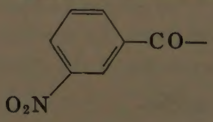
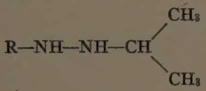
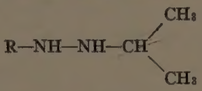
	191		134
	111		123
	0		97
	152		76
	0		63
	192		11
	84		0
	0		15
	38		60
	38		125

TABLE 2

Amino acid derivatives		Aliphatic acid derivatives	
			
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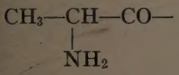
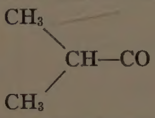
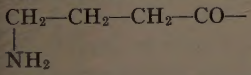
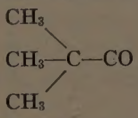
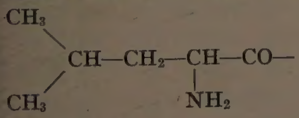
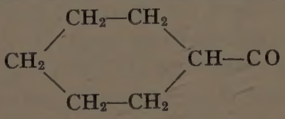
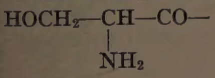
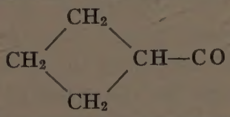
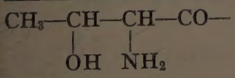
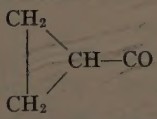
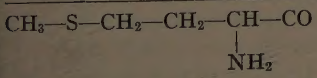
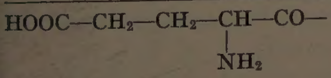
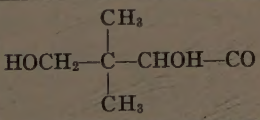
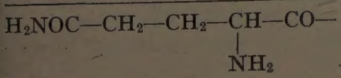
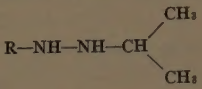
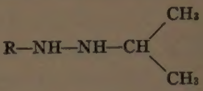
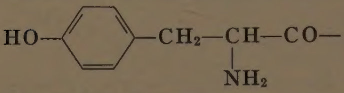
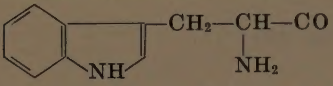
 $CH_3—\underset{NH_2}{CH}—CO—$	253	HCO—	138
$H_2N—CH_2—CH_2—CO—$	21		97
 $\underset{NH_2}{CH_2}—CH_2—CH_2—CO—$	13	$CH_3—CHOH—CO—$	83
$CH_3—NH—CH_2—CO—$	47		152
	200		147
 $HOCH_2—\underset{NH_2}{CH}—CO—$	189		143
 $CH_3—\underset{OH}{CH}—\underset{NH_2}{CH}—CO—$	133		91
 $CH_3—S—CH_2—CH_2—\underset{NH_2}{CH}—CO$	147	$CH_3—CH{=}CH—CO—$	74
 $HOOC—CH_2—CH_2—\underset{NH_2}{CH}—CO—$	250	 $HOCH_2—\overset{CH_3}{\underset{CH_3}{C}}—CHOH—CO$	2
 $H_2NOC—CH_2—CH_2—\underset{NH_2}{CH}—CO—$	194	$CH_3O—CH_2—CO—$	178

TABLE 2 —*continued*

Amino acid derivatives		Aliphatic acid derivatives	
			
R =	Marsilid index	R =	Marsilid index
	195	$CH_3—SO_2—CH_2—CO—$	0
	137	$(C_2H_5O)_2CH—CO—$	221

an acyl group. Activity drops, going from an α-amino acid such as alanine to β-alanine or γ-aminobutyric acid. Among the more potent hydrazides are those of leucine, serine, glutamic acid, and tyrosine. In the aliphatic series pivalic acid and some alkoxy-substituted acids show high activity.

The methods of synthesis most often used are shown in tabular form (TABLE 3).

TABLE 4 shows the results of attempts to find the optimal size of the alkyl group on the hydrazide part of the molecule. Serine derivatives with an alkyl group consisting of 3 or 4 carbon atoms show the highest activity; this was confirmed in a comparable series with other acyl radicals.

TABLE 5 shows a comparison of activity between derivatives of pivalic acid with isopropyl and araliphatic groups. Whereas some of the araliphatic derivatives are more potent than the simple alkyl derivatives, substitution on the benzene ring diminishes the activity.

The same is true for the series of compounds derived from *p*-chlorobenzoic acid shown in TABLE 6. Replacement of the benzene ring by a pyridine ring leads to reduced potency.

In TABLE 7 the activities of the isopropyl and benzylhydrazine derivatives of some acids are compared. In some cases, such as with the pivalic acid derivatives, there is no difference between the two compounds, but with other acids either the isopropyl or the benzyl compounds are the more active ones.

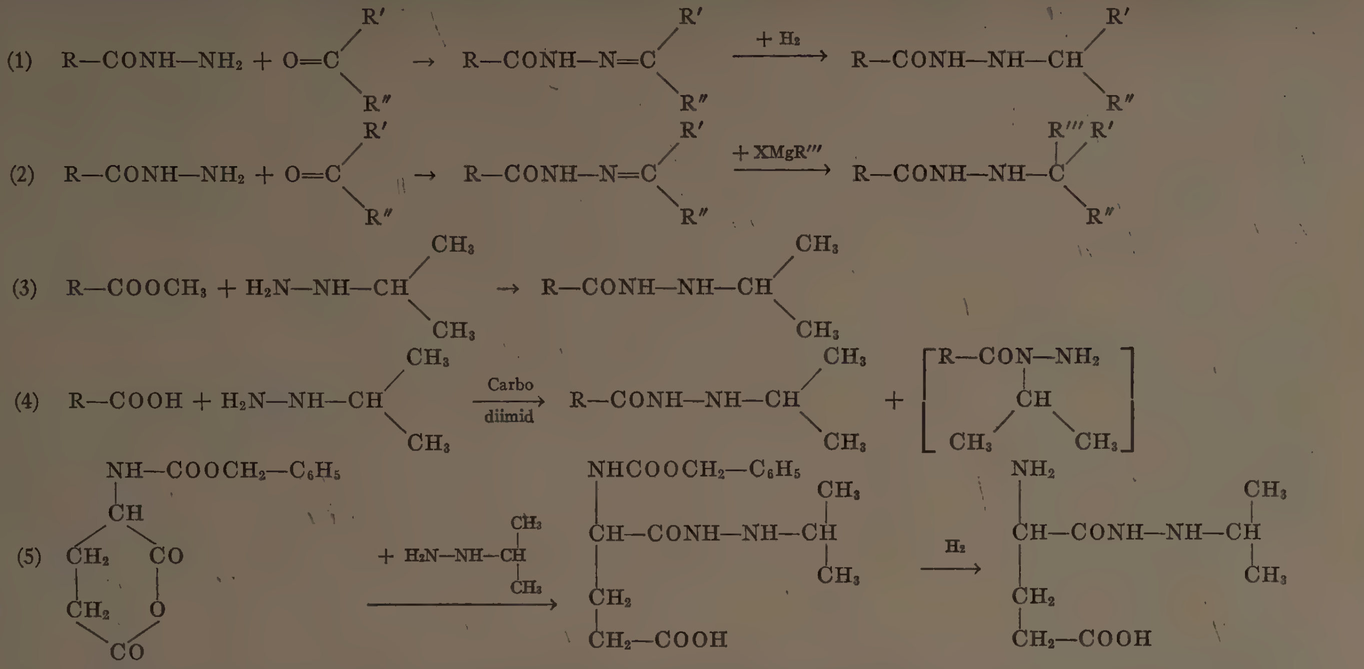
From these and other results it was not possible to discover which group, if any, on the hydrazine part gives maximal activity. It seems to be the combination of both parts of the molecule that determines potency, and the most active compound in a given series must be found experimentally.

Introduction of a further isopropyl group on the second nitrogen atom of the hydrazine part of the molecule leads to compounds with enhanced activity in some cases (TABLE 8) but, again, no regularity can be observed.

Some observations made with amino acid derivatives deserve special men-

TABLE 3

METHODS OF PREPARATION OF AMINE OXIDASE INHIBITORS*



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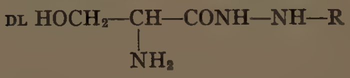
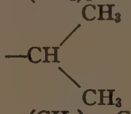
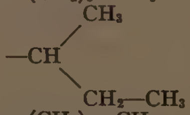
* (1) The reaction used most frequently, a hydrazide reacting with a carbonyl compound followed by hydrogenation of the hydrazone; (2) addition of a Grignard reagent to a hydrazone produces tertiary hydrazides; (3) reaction of a methyl ester with isopropylhydrazine gives somewhat irregular results, but proved of value in selected cases; (4) the application of a carbodiimide was especially useful in the amino acid field, where mild reaction conditions are necessary to prevent racemization; and (5) a special method that was necessary to obtain compounds such as the α-isopropylhydrazine derivative of glutamic acid.

tion. From the data in TABLE 9 it is obvious that for the amino acid derivatives there are marked differences in activity when measured *in vitro* or *in vivo*, but these do not appear with iproniazid or the simple isopropylhydrazine. This suggests that the hydrazides of amino acids are involved either in some transport phenomena or are metabolized by pathways not used for iproniazid. Active transport against concentration gradients has been demonstrated for amino acids, especially in nerve tissues. The higher activity *in vivo* might therefore be explained by an enhanced transport through some physiological barrier. Attention should be drawn to the fact that both the free methionine derivative and the *N*-acetylated compound have comparable activity *in vivo*, but not *in vitro*. This effect has been observed also with other amino acids and is probably due to hydrolysis occurring *in vivo*, but not to the same extent *in vitro*. In addition to the above-mentioned peculiarities, striking differences of activity between the natural L- and the unphysiological D-forms of the amino acid derivatives have been observed (TABLE 10).

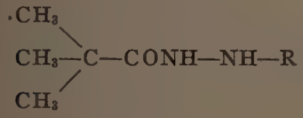
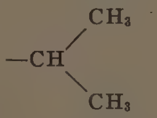
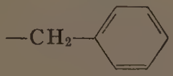
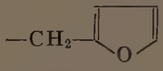
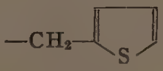
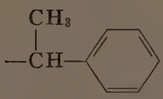
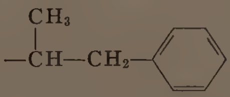
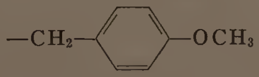
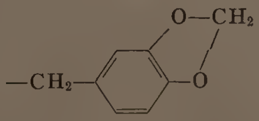
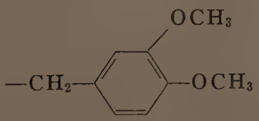
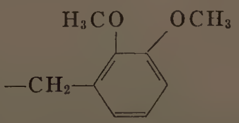
TABLE 4

Formula	R	Marsilid index
DL  $HOCH_2\text{—}\underset{NH_2}{\underset{\vert}{CH}}\text{—}CONH\text{—}NH\text{—}R$	$—CH_3$	30
	$—CH_2—CH_3$	128
	$—(CH_2)_2—CH_3$	97
		189
	$—(CH_2)_3—CH_3$	187
		80
	$—(CH_2)_4—CH_3$	148
	$—(CH_2)_5—CH_3$	95
	$—(CH_2)_6—CH_3$	33
	$CH_2—CH_3$ $—(CH_2)_7—CH_3$ $CH_2—CH_2—CH_3$	0

For example, L-alanine isopropylhydrazide is highly active, whereas the D-form is nearly inactive, and the racemate is in between. This effect is less marked with serine, pyroglutamic acid, and the γ-derivative of glutamic acid, and is absent altogether with leucine and phenylalanine. Making the amino acids even more unnatural, that is, by introducing methyl groups on the nitrogen atom, which is known to produce antimetabolites, completely abolishes the activity, as may be seen in TABLE 11, with examples of alanine and serine derivatives.

The data from the foregoing tables indicate that, by variation of both the acyl and alkyl groups, marked differences of activity, at least in brain, may be obtained, but such variations also lead to compounds with different activities in selected organs. Thus, the hypothesis underlying the present research program obtains confirmation from the data in TABLE 12. This table shows the differences of activity of two selected hydrazides for such organs as brain and heart. For brain the Marsilid index as previously defined is given, but for the heart the increase of norepinephrine (instead of 5-hydroxytryptamine) is taken as an index of MAO inhibition, the values for iproniazid being fixed as 100 in both columns; the ratio of brain to heart therefore becomes 1 by

TABLE 5

Formula	R =	Marsilid index
		152
		156
		0
		141
		210
		84
		113
		78
		46
		15

definition. The glutamic acid derivative now has a heart value that is lower and a brain value that is higher than the corresponding iproniazid values, thus bringing the ratio to 3.3. On the other hand, the values for the palmitic acid derivative are just reversed, giving a ratio of 0.4. Thus, compared to iproniazid, the isopropylhydrazide of glutamic acid has a greater activity in the brain than in the heart, whereas the isopropylhydrazide of palmitic acid has a rela-

TABLE 6

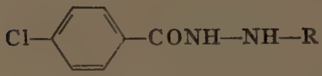
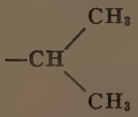
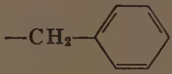
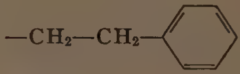
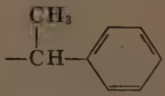
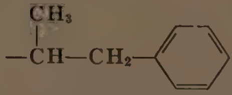
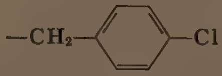
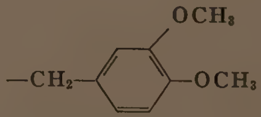
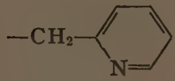
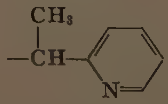
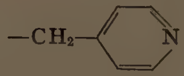
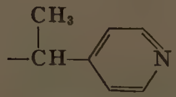
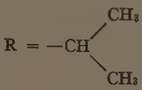
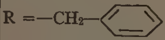
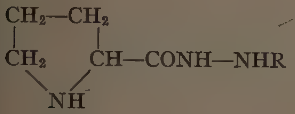
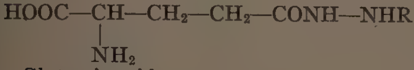
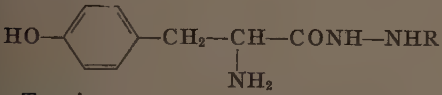
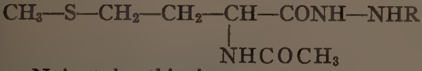
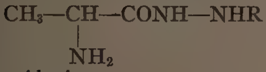
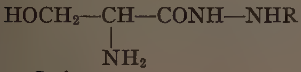
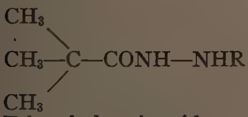
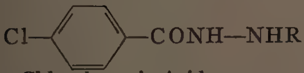
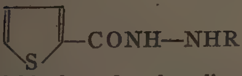
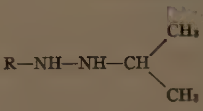
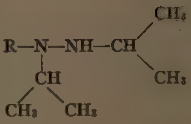
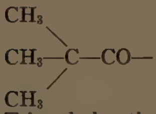
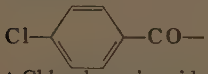
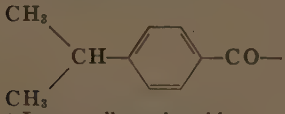
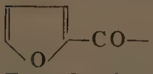
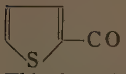
Formula	R =	Marsilid index
		136
		219
		106
		203
		54
		68
		27
		19
		19
		60
		49

TABLE 7

Formula	Marsilid index	
	 $R = —CH{<}^{CH_3}_{CH_3}$	
 L-Proline	174	136
 $HOOC—\underset{NH_2}{CH}—CH_2—CH_2—CONH—NHR$ L-Glutamic acid	178	151
 L-Tyrosine	162	184
 $CH_3—S—CH_2—CH_2—\underset{NHCOCH_3}{CH}—CONH—NHR$ DL-N-Acetylmethionine	143	100
 $CH_3—\underset{NH_2}{CH}—CONH—NHR$ L-Alanine	253	157
 $HOCH_2—\underset{NH_2}{CH}—CONH—NHR$ DL-Serine	192	173
 Trimethylacetic acid	152	156
$CH_3—CHOH—CONH—NHR$ Lactic acid	83	214
 *p*-Chlorobenzoic Acid	136	220
 Thiophene-2-carboxylic acid	198	133

tively greater activity in the heart. Thus, some progress in developing MAO inhibitors with a selective affinity for a certain organ has been made, at least in experiments on animals. Clinical evidence must be sought to determine whether this effect can be demonstrated also in humans.

TABLE 8

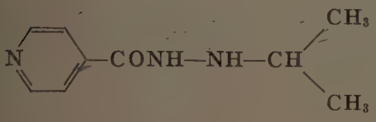
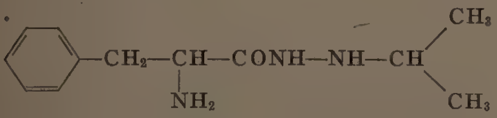
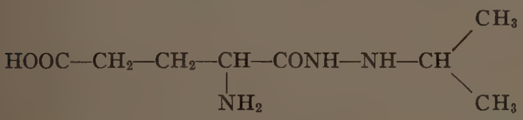
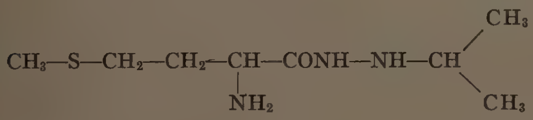
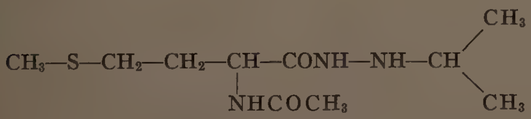
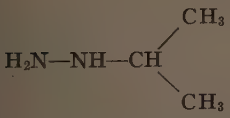
R =	Marsilid index	
		
$CH_3—CO—$ Acetic Acid	74	256
 Trimethylacetic acid	152	112
$CH_3—(CH_2)_{14}—CO—$ Palmitic acid	63	22
$CH_3CH—OH—CO—$ Lactic acid	83	196
 *p*-Chlorobenzoic acid	136	70
 *p*-Isopropylbenzoic acid	97	138
 Furan-2-carboxylic acid	156	116
 Thiophene-2-carboxylic acid	198	24

Discussion of the Paper

J. A. AESCHLIMANN (*Hoffmann-La Roche Inc. Nutley, N. J.*): Zeller *et al.* are to be congratulated on the enormous amount of painstaking work carried out in synthesizing and investigating the action of such a large group of hy-

TABLE 9

INHIBITION OF MONOAMINE OXIDASE OF RAT BRAIN *IN VITRO* AND *IN VIVO* BY AMINO ACID ISOPROPYLHYDRAZIDES AS COMPARED TO IPRONIAZID

	In Vitro	*In Vivo*
	Inhibition of monoamine oxidase* relative to Marsilid	Increase of 5HT in rat brain 16 hours after i.p. application
	(concentration of inhibitors 3×10^5 M/L)	Equimolar dose to 100 mg./kg. iproniazid
 iproniazid	100 ± 5	100 ± 6
L 	44 ± 5	171 ± 3
L  $HOOC-CH_2-CH_2-CH(NH_2)-CONH-NH-CH(CH_3)_2$	59 ± 8	227 ± 21
DL  $CH_3-S-CH_2-CH_2-CH(NH_2)-CONH-NH-CH(CH_3)_2$	57 ± 6	147 ± 4
DL  $CH_3-S-CH_2-CH_2-CH(NHCOCH_3)-CONH-NH-CH(CH_3)_2$	29 ± 8	143 ± 13
 $H_2N-NH-CH(CH_3)_2$	≥200	200 ± 13

* Tyramine oxidation by brain mitochondria.

TABLE 10

Amino acid isopropylhydrazides	Marsilid index		
	L-Form	D-Form	Racemate
Alanine	253	22	130
N-Acetylalanine	210	0	123
Leucine	188	—	214
Phenylalanine	171	—	168
Serine	213	133	192
Glutamic acid α-derivative	250	0	135
Glutamic acid γ-derivative	178	76	154
Pyroglutamic acid	134	—	97

drazides. In particular, they investigated one aspect of this activity: the increased levels of 5-hydroxytryptamine in the brain and liver after the administration of these compounds. This did not necessarily parallel the *in vitro* inhibition of amine oxidase. It is noteworthy that the unnatural amino acid hydrazides had a lower activity than the hydrazides of the naturally-occurring acids that could be explained by a difference in the degradation or

TABLE 11

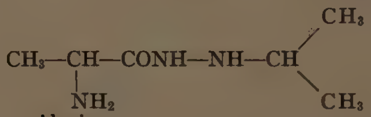
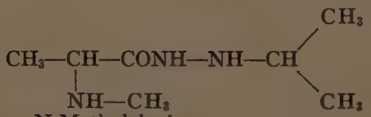
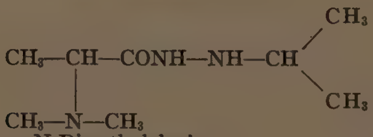
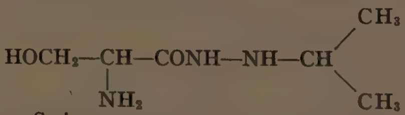
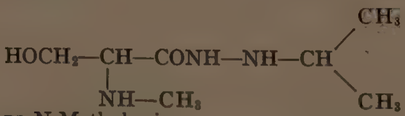
Formula	Marsilid index
 DL-Alanine	130
 DL-N-Methylalanine	0
 DL-N-Dimethylalanine	8
 DL-Serine	189
 DL-N-Methylserine	6

TABLE 12

Isopropylhydrazide of	Percentage increase of monoamines		Ratio
	Heart catecholamines	Brain 5-HT	
Isonicotinic acid (Marsilid)	100	100	1.0
Glutamic acid (α-derivative)	75	250	3.3
Palmitic acid	145	60	0.4

utilization. However, unphysiological acids, such as chlorobenzoic acid, formed hydrazides that were about equally active with those of palmitic acid, which is a normal constituent of the diet and is readily broken down. The operative sentence summarizing the authors' work is, "From these and many other results we were unable to find an indication as to which group on the hydrazine part gives highest activity." If the authors had been content to make only a few compounds, they might have been able to fit them into a theory that would have sounded logical until the work had been extended. The irregularity of the observations makes it difficult to comment on the results obtained.

STRUCTURE AND ACTIVITY RELATIONSHIPS OF MONOAMINE OXIDASE INHIBITORS

John H. Biel, Patrick A. Nuhfer, Alvin C. Conway
Lakeside Laboratories, Inc., Milwaukee, Wis.

The advent of the tranquilizing drugs brought about a definite change in the philosophy of the treatment of mental disease.

At least two benefits accrued from this change in our thinking: (1) it pointed a way toward the eventual physiological treatment of mental disease, and (2) it raised the possibility that certain therapeutic concepts and techniques that had dealt successfully with diseases other than mental could be extended and applied in the treatment of psychiatric disorders.

The concepts to which I refer concern themselves primarily with the roles of such familiar chemical mediators of normal and abnormal physiological functions as acetylcholine, norepinephrine, epinephrine, histamine, and serotonin, as well as with the variety of approaches of potentiating and inhibiting their pharmacological effects.

In other words, by chemically manipulating these endogenous substances we had been able to hand-control certain bodily processes that had begun to malfunction because of a breakdown in the once carefully balanced automatic control mechanism (homeostatic equilibrium) of the body.

Diseases such as peptic ulcer, ulcerative colitis, biliary disorders, hypertension, allergic manifestations, and asthma could be treated in this fashion, at least symptomatically.

The work of Brodie,[1] Woolley,[2] Gaddum,[3] and Mills and Slater[4] indicated that similar physiological mechanisms might be operating in the brain, and that their potentiation or inhibition might result in a practical approach to the treatment of mental disease.

In the light of these concepts I shall review briefly the subject of central stimulants and then present in greater detail some of the newer approaches to the chemotherapy of the depressive state that are being attempted in our laboratories.

The status of the central stimulants up to 1955 was perhaps adequately summed up by Goodman and Gilman[5] in the second edition of *The Pharmacological Basis of Therapeutics*: "Although central nervous system stimulants are sometimes dramatic in their pharmacological effects, they are relatively unimportant from a therapeutic point of view. It is not possible to stimulate the central nervous system for a long period of time, for heightened nervous activity is followed by depression, proportional in degree to the intensity and duration of the stimulation. Consequently, therapeutic excitation of the central nervous system is usually of brief duration and is reserved for emergencies characterized by severe central depression."[5] This statement was based, of course, on the action of those drugs available at the time for stimulation of the central nervous system (CNS). Such agents are picrotoxin, metrazol, strychnine, camphor, nikethamide, and caffeine.

Picrotoxin and metrazol were used most often in respiratory depression resulting from barbiturate poisoning. Metrazol and camphor were, of course, the forerunners to the electroconvulsive therapy of schizophrenia. The first four of these drugs produce central excitation only at the convulsive dose, the action of nikethamide is similar to that of picrotoxin, but it is a much less potent agent.[6]

The central stimulant properties of caffeine are well known. However, this compound is of no value in the treatment of the emotionally depressed state.

TABLE 1

PHARMACOLOGICAL AREAS IN WHICH CENTRAL STIMULANTS HAVE BEEN FOUND

Sympathomimetics:	amphetamine, methamphetamine, ephedrine
Antihistamines:	phenyltoxamine and G-22355
Anticholinergics:	atropine and related compounds
Local anesthetics:	cocaine
Ganglionic stimulants:	nicotine, lobeline

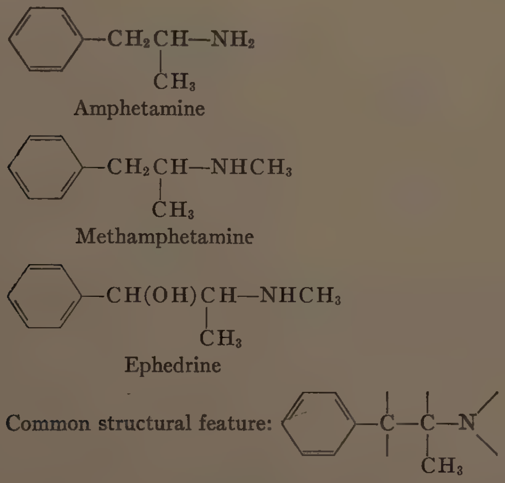


FIGURE 1. Sympathomimetic amines.

The second group of compounds that act on the CNS is found among the sympathomimetic agents, modified antihistamines, anticholinergic compounds, local anesthetics, and ganglionic stimulants (TABLE 1). I mention this group separately, since some of its members have become important starting points in the development of newer agents in this field.

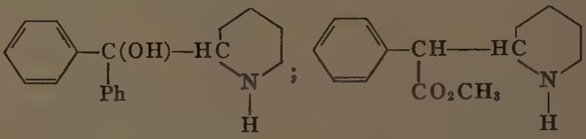
Amphetamine, methamphetamine, and ephedrine belong to the important class of phenylalkylamines (FIGURE 1).

Several of the newer stimulants contain the phenethylamine skeleton (FIGURE 2).[6-9]

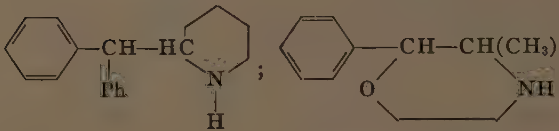
Variation of certain antihistaminic structures has not only yielded potent tranquilizers, but also a number of central stimulants (FIGURE 3).[10-12]

A number of potent anticholinergic agents that are congeners of atropine (FIGURE 4) are also potent central stimulants both in animals and man.[13-17]

Cocaine and the *p*-acetylaminobenzoic acid salt of dimethylaminoethanol (Deaner) both produce central stimulatory effects (FIGURE 5).[18, 19] Deaner has proved useful in the treatment of mild depressions.[20] Pfeiffer[19] ascribes its action to the fact that dimethylaminoethanol, a precursor of acetylcholine, can readily penetrate the blood-brain barrier and is converted in the brain to

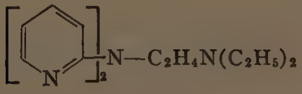


Pipradol (Meratran) Methyl phenidate (Ritalin)



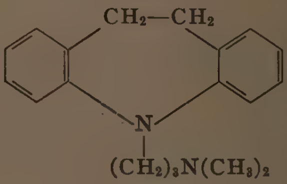
Phenmetrazin (Preludin)

FIGURE 2. Central stimulants with β-phenylethylamine skeleton: modified phenylethylamines.[6]

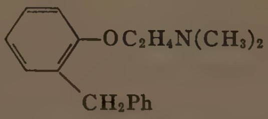


IN-33

3.0 mg./kg. causes alertness in dogs (salivation, mydriasis)

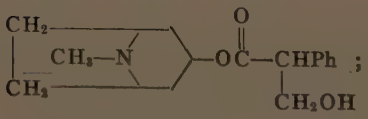


G-22355

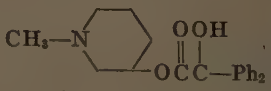


Phenyltoloxamine

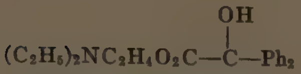
FIGURE 3. Modified antihistamines as central stimulants.



Atropine



JB-336



$$(C_2H_5)_2NC_2H_4O_2C-\overset{OH}{\overset{|}{C}}-Ph_2$$

Benactyzine (Suavitil)

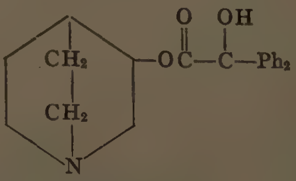
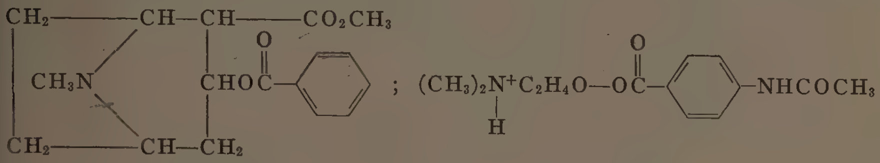


FIGURE 4. Anticholinergics as central stimulants.

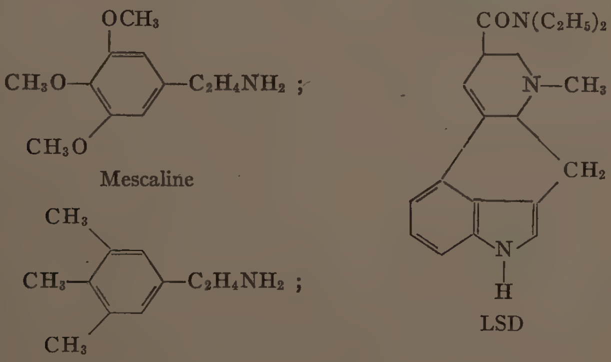
acetylcholine. The increased amounts of brain acetylcholine are said to produce an antidepressant effect.

The central effects of mescaline and lysergic acid diethylamide (LSD) have been adequately reviewed by Koelle,[21] by Stoll,[22] and by Rothlin.[23] It is interesting to note that the trimethyl analogue of mescaline appears to exert similar actions[24] (FIGURE 6).


Cocaine

Deaner

FIGURE 5. Local anesthetics as central stimulants.


Mescaline

LSD

FIGURE 6. Hallucinogenic compounds.

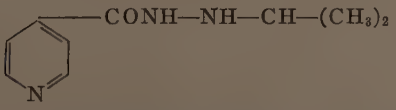

Iproniazid (Marsilid)

FIGURE 7. Monoamine oxidase (MAO) inhibitor. MAO inhibition was first described by Zeller.[28]

From these few examples it becomes evident that the majority of the central stimulants presently available embody within their molecular architecture certain structural features contained in norepinephrine, acetylcholine, histamine, and serotonin, and that by mimicking, potentiating, or inhibiting the physiological effects of these endogenous amines it might be possible to arrive at a chemical therapy of the depressed state. Brodie [1, 25] has pointed out that central stimulation could be achieved by the potentiation of the adrenergic centers or the inhibition of the cholinergic centers of the hypothalamus.

Additional proof for this hypothesis comes from the monoamine oxidase (MAO) inhibitors such as iproniazid, which prevent the destruction of endogenous excitatory amines.[26, 27] The potent MAO inhibitory properties of iproniazid (FIGURE 7) both *in vivo* and *in vitro* were first discovered by Zeller.[28-30]

We entered the field of central stimulants in the course of a broad program dealing with the structure-activity relationships of a new series of bronchodilators.[31] We were impressed at the time with the evanescent action of many of the sympathomimetic amines, due in part to the oxidative enzymatic degradation of the aminoalkyl side chain by MAO[32-36] (FIGURE 8). One means of obtaining a more stable sympathomimetic agent while still preserving the potent bronchodilator activities of epinephrine was to replace the N-methyl group with certain substituted aralkyl moieties (FIGURE 9). However, this approach did not appear fruitful with respect to the other useful actions of these amines, that is, their cardiovascular and CNS effects.

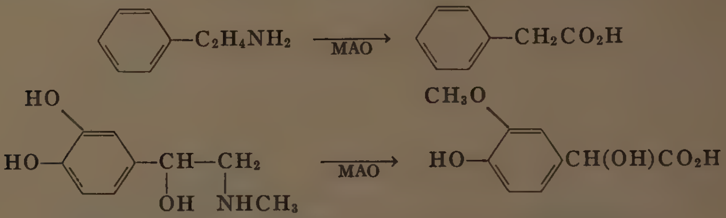
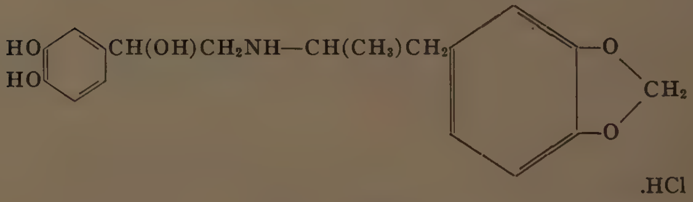


FIGURE 8. Monoamine oxidase action on β-phenylethylamines.



JB-251 (Caytine)

FIGURE 9. The orally active bronchodilator JB-251 (Caytine). This drug is orally effective in man at 2.5-mg. dosage for 3 to 4 hours.

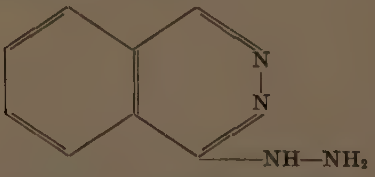


FIGURE 10. Hydralazine (Apresoline).

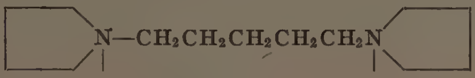
We turned to hydrazine analogues of the sympathomimetic amines for a variety of reasons.

First, we hoped that the hydrazines would have a greater affinity for the cell receptor sites and would not be subject to the same type of enzymatic degradation. In this way a more intense and prolonged action could be expected.

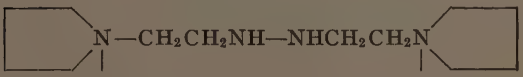
Second, certain hydrazine derivatives such as hydrazinophthalazine (Apresoline—see FIGURE 10) had been shown to be useful agents for lowering blood pressure,[26, 38] and the incorporation of a hydrazine group into a skeleton with an already great affinity for the blood pressure-regulating centers appeared to be a logical approach to the development of agents for the "manual" control of blood pressure disturbances. We were encouraged further in this approach by our finding that the replacement of a methylene by a hydrazino group in a number of *bis*-onium hypotensive agents greatly potentiated the intensity and duration of the hypotensive effect (FIGURE 11).[39]

Finally, we were interested in testing the isosteric effect of replacing a methyl or methylene by an NH_2 or NH grouping on biological activity.

Hence, we proceeded with the synthesis of sympathomimetic hydrazines where the hydrazine moiety served to replace an amino, methylamino, or methyleneamino grouping (TABLE 2). The type of series chosen for synthesis resembled closely that of Barger and Dale[40] for the sympathomimetic amines. In their classic work these investigators showed that the nature of the physiological response elicited by the phenylalkyl amines was dependent on: (1) the nature of the nuclear substituent R, (2) the nature and length of the side chain Y, and (3) the type of substituent on the amino nitrogen (R_1 and R_2). This is shown in FIGURE 12.



10 mg./kg. (p.o.)/−6/65′

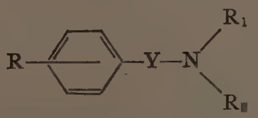


10 mg./kg. (p.o.)/−28/300′

FIGURE 11. Hydrazine analogues of *bis*-onium hypotensives.

TABLE 2

Sympathomimetic amine	Hydrazine analogue
$PhCH_2CH_2NH_2$	$PhCH_2NH{-}NH_2$
$PhCH_2CH(CH_3)NH_2$	$PhCH_2N(CH_3)NH_2$
$PhCH_2CH(CH_3)NH{-}CH_3$	$PhCH_2CH(CH_3)NH{-}NH_2$



R = Nuclear substituent(s)
Y = Alkylene chain
R_1; R_2 = H or alkyl

FIGURE 12. Sympathomimetic amines.

We now propose to show: (1) similar structure-activity relationships among the sympathomimetic hydrazines with respect to CNS stimulation and MAO inhibition; (2) the alteration in physiological response evoked by replacing an "amino" with a "hydrazino" group; and (3) the increased intensification in the MAO-inhibitory properties of the hydrazine derivatives as compared to the parent amines. Certain hydrazine derivatives had previously been prepared and tested by Bovet[41] and claimed to have pressor activities comparable to the amine congeners (FIGURE 13).

Structure-Activity Relationships

It had previously been demonstrated by Zeller[28] that N-isopropyl-N′-isonicotinoyl hydrazine (iproniazid) is a potent inhibitor of MAO. Hence, the compounds were screened for both their MAO inhibitory effects and their analeptic properties. The *in vivo* work was done under the supervision of one of us (P.A.N.) in our Pharmacology Division. The *in vitro* enzyme studies were performed by A. Horita of the School of Medicine, University of Washington, Seattle, Wash. We are indebted to these investigators for the use of their data.

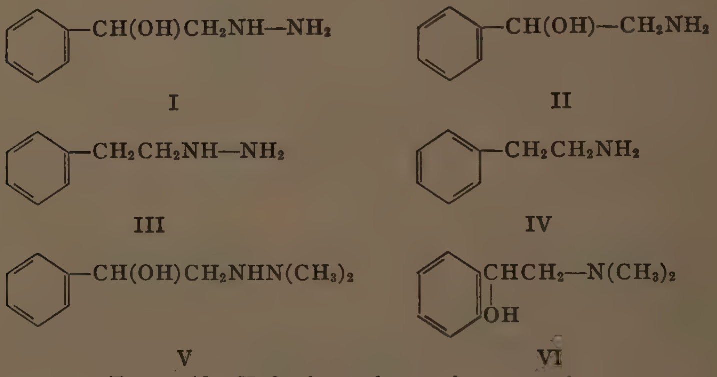


FIGURE 13. Hydrazine analogues of pressor amines.

Pletscher *et al.*,[42, 43] as well as Carlsson *et al.*,[44] have shown that reserpine is a potent releasing agent of such endogenous monoamines as serotonin, epinephrine, norepinephrine, and dihydroxyphenylethylamine (dopamine) from their binding sites and that, in the presence of an MAO inhibitor, these amines are not metabolized and thus reverse the sedative action of reserpine, provided the enzyme inhibitor is given prior to the administration of reserpine.[45] Hence the threshold dose of the MAO inhibitor capable of reversing the reserpine sedation was taken as a measure of the *in vivo* enzyme inhibitory potency. Iproniazid was taken as the standard, and the potency of the new compounds was expressed in terms of iproniazid activity. A compound having less than twice the potency of iproniazid was not followed further for its ultimate activity in this screening test.

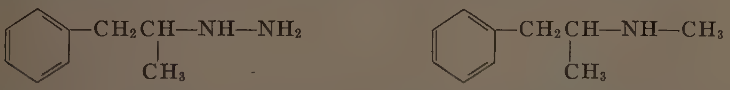
The *in vitro* potency of the drugs was determined by Horita by incubating rat-liver homogenate with serotonin as the substrate.[46] The inhibitors were added to the mixture at concentrations of 10^{-5} M and 10^{-6} M before incubation with the substrate. The degree of inhibition was expressed as percentage decrease in ability to metabolize the substrate as compared to control samples.

The analeptic potency (TABLES 3 to 8) of the drugs was measured by administering reserpine first (5.0 mg./kg. intraperitoneally to mice) and then determining what nontoxic dose of the test drug could arouse the animal from its reserpine stupor. If the compound was a "pure" MAO inhibitor, it was unable to reverse the sedative effect of reserpine. However, if it possessed analeptic properties of its own, a reserpine reversal could be demonstrated. This type of action would be comparable to that of amphetamine or Ritalin.[45, 47] The structure-activity data are summarized in TABLES 3 and 5 to 8.

The potent MAO inhibitory properties for one of the compounds of this series, 1-phenyl-2-hydrazinopropane (JB-516—see FIGURE 14), have previously been described by Horita.[46] Shore *et al.*[48, 49] also showed that this compound was capable of raising serotonin and norepinephrine brain levels in the rabbit twofold to threefold at one tenth the dose of iproniazid.

The effect of chain length on the MAO inhibitory and analeptic properties of the aralkyl hydrazines is shown in TABLE 3.

Maximum MAO inhibition and analeptic activity were obtained with the amphetamine side chain (No. 7). These data correlated well with the *in vitro* data of Horita.[46] While the benzyl hydrazine (No. 2) was a potent enzyme inhibitor, it displayed analeptic activity only at the convulsant dose. Shifting of the methyl group to the β-carbon (No. 8) resulted in a 50 per cent loss in activity. Removing the methyl group from the phenethyl side chain (No. 3) produced a tenfold decrease in enzyme inhibitory potency and a loss of the analeptic activity. The α-phenethyl hydrazine (No. 4), on the other hand, was still a potent enzyme inhibitor, although the lengthening of the branched alkyl chain (No. 6) brought about a steep fall in the activity. Replacement of methyl by isosteric hydroxyl (No. 4 versus No. 5) produced an inactive compound. Lengthening the side chain to butyl (No. 9) reduced *in vivo* enzyme inhibitory activity by 50 per cent (compare No. 7) and abolished the analeptic properties. Iproniazid also was devoid of analeptic activity.



JB-516 Methamphetamine

FIGURE 14. Comparison of JB-516 with methamphetamine. JB-516 may be considered a nitrogen isoster of methamphetamine (desoxyphedrine).

Similar structure-activity relationships exist in the phenylalkyl amine series.

Potent sympathomimetic activities are displayed only with the β-phenethylamines according to Barger and Dale[40] (TABLE 4). Similarly, enzyme inhibition becomes marked with the nitrogen isoster of phenethylamine: benzyl hydrazine (No. 2). Only 1-phenyl-2-propylamine has analeptic properties. Any changes in the structure abolished this activity.[50] The analeptic properties of amphetamine and *N*-aminoamphetamine are quite comparable (No. 12 and No. 7).

The influence of nuclear substitution on the enzyme inhibitory and analeptic properties is shown in TABLE 5.

Progressive methoxylation progressively decreases MAO inhibition and abolishes analeptic activity (Nos. 13, 14, and 15), yet the 3,4-methylenedioxy derivative (No. 16) is still a very potent compound in both respects. Alkylation likewise reduced both types of activities (Nos. 17 and 18). The chloro derivative (No. 19) was the most potent compound in this series. Hydrogenation of the ring (No. 20) reduced enzyme inhibitory action considerably, but still yielded a compound with potent analeptic properties (TABLE 5).

Similar relationships are encountered in the sympathomimetic series where methylation (one sixth of the CNS stimulant activity of amphetamine), methoxylation, and hydrogenation reduce or abolish the CNS effects of amphetamine.[51-53]

TABLE 3

EFFECT ON CHAIN LENGTH ON MAO INHIBITION

$Ph{-}Y{-}NH{-}NH_2$

No.	Y	*In vivo** potency (Marsilid = 1)	*In vitro* per cent of inhibition		Analep.† mg./kg.	LD_{50}mg./kg. (s.c., mice)
			10^{-5} M	10^{-6} M		
1	—	<1			None	
2	CH_2—	40	100	21	60‡	68
3	CH_2CH_2—	4	50	30	None	102
4	$\underset{\substack{\mid \\ CH_3}}{CH}{-}$	20			None	
5	$\underset{\substack{\mid \\ OH}}{CH}{-}CH_2{-}$	8	0		None	
6	$\underset{\substack{\mid \\ C_2H_5}}{CH}{-}$	<2	65	5	None	
7	$CH_2{-}\underset{\substack{\mid \\ CH_3}}{CH}{-}$	40	100	60	1.0	95
8	$\underset{\substack{\mid \\ CH_3}}{CH}{-}CH_2$	16	80	25	None	
9	$CH_2CH_2\underset{\substack{\mid \\ CH_3}}{CH}{-}$	20	100	80	None	
11	Iproniazid	1	25	0	None	
12	Amphetamine				1.0	20

* Drug administered to mice intraperitoneally 2 hours prior to the intraperitoneal administration of 5.0 mg./kg. of reserpine.

† Analeptic activity: 5.0 mg./kg. (intraperitoneally) of reserpine administered to mice 3 to 4 hours prior to the administration of the drug.

‡ Convulsions occurred at this dosage.

TABLE 4

	Pressor activity	Analeptic activity
$PhNH_2$	None	0
$PhCH_2NH_2$	Slight	0
$Ph(CH_2)_2NH_2$	Potent	0
$PhCH_2CH(CH_3)NH_2$	Potent	Potent
$PhCH(CH_3)CH_2NH_2$	Potent	0
$Ph(CH_2)_3NH_2$	Weak	0

N-acylation substantially decreased MAO inhibition and abolished the potent analeptic properties of phenylisopropyl hydrazine in the initial screening test (TABLE 6).

The effect of nitrogen substitution is illustrated further in TABLE 7. N-alkylation decreased both enzyme inhibitory and analeptic potency. Most interesting is the fact that the N,N-dimethyl compound (No. 25) is a more potent MAO inhibitor than the monomethyl derivative (No. 24). While N-methyl amphetamine is claimed to be somewhat more active as an analeptic, N,N-dimethyl amphetamine has only one thirtieth the CNS stimulant effect of the parent compound.

TABLE 5

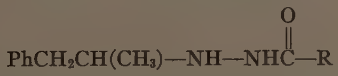
EFFECT OF NUCLEAR SUBSTITUTION

$R—Ph—CH_2CH(CH_3)NH—NH_2$

No.	R	*In vivo* potency (Marsilid = 1)	*In vitro* potency percentages of inhibition		Analeptic activity mg./kg.	LD_{50} mg./kg.
			10^{-5} M	10^{-6}M		
13	p-OCH_3	8	100	10	10(?)	118
14	3,4-$(OCH_3)_2$	4	65	15		130
15	3,4,5$(OCH_3)_3$	2	50	5		100
16	3,4-CH_2O_2	20	100	55	15	100
17	*o*-Methyl	20	100	45	12	114
18	*p*-Isopropyl	4	95	25		108
19	*m*-Chloro	20	100	49	3	84
20	6-H	4	86		8	
	Iproniazid	1	25	0		

TABLE 6

EFFECT OF N-ACYL SUBSTITUTION



$$PhCH_2CH(CH_3)—NH—NH\overset{\overset{\displaystyle O}{\|}}{C}—R$$

No.	R	*In vivo* potency (Marsilid = 1)	*In vitro* potency percentage of inhibition		Analeptic activity mg./kg.	LD_{50} mg./kg.
			10^{-5} M	10^{-6} M		
21	CH_2CN	2			None	
22	4-Pyridyl	4	50	0	None	
23	$Ph_2C(OH)$	2			None	Fatal at 80 mg./kg.

The N-isopropyl derivative (No. 26) appeared to be only a moderately active compound. However, Spector *et al.*[54] demonstrated that, 24 hours after the administration of the drug, this compound was as potent as phenylisopropyl hydrazine (No. 7 in TABLE 3) in raising serotonin and norepinephrine levels in rabbits. However, no symptoms of central excitation were observed in contrast to the potent CNS-stimulating properties of the unsubstituted derivative. The mode of action of this compound is now under intensive investigation, since it may throw some light on the mechanism of CNS stimulation of the MAO inhibitors. It is possible that the drug may block the receptor sites for the excitatory catechol amines (dopamine and norepinephrine). Its delayed action could also suggest metabolic degradation to a more active MAO inhibitor.

Table 7

Effect of *N*-Alkylation

$PhCH_2—CH(CH_3)—N(R_1)—N(R_2)(R_3)$

No.	R_1	←R_2	←R_3	*In vivo* potency (Marsilid = 1)	*In vitro* percentages of inhibition		Analeptic activity mg./kg	LD_{50} mg./kg.
					10^{-5} M	10^{-6} M		
24	H	H	CH_3	3			10	126
25	H	CH_3	CH_3	8			15	109
26	H	H	$CH(CH_3)_2$	2			50	269
27	H	CH_2SO_3Na		16	100			
28	H	H	CH_2SO_3H	20				
29	H	1,2,3,4 THIQ*		2			None	
31	H	H	$CH(CH_3)CH_2Ph$	2	12	0		
32	$PhCH_2N(CH_3)—NH_2$			2	6	0	None	

* $N(R_2)$ (R_3) = Tetrahydroisoquinolino.

Table 8

Effects of Heretocyclic Rings

$R—CH_2NH—NH_2$

No.	R	*In vivo* potency (Marsilid = 1)	*In vitro* potency percentages of inhibition		Analepsis
			10^{-5} M	10^{-6} M	
33	2-Pyridyl		15		None
34	3-Pyridyl	4	73		None
35	2-Thienyl	8	57		None
36	2-Furyl				None
37	2-Benzodioxan	5			None
	Phenyl	40			(?)

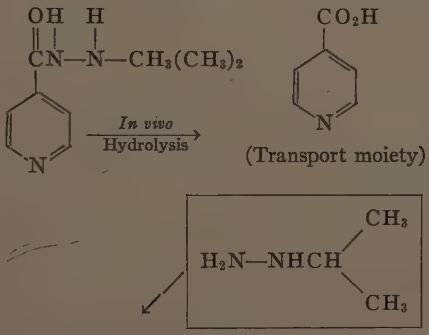
Replacement of the phenyl by several heterocyclic ring systems substantially reduced MAO inhibitory activity (TABLE 8). Similarly, heterocyclic derivatives of amphetamine display little, if any, analeptic properties.[55-58]

The cardiovascular properties of JB-516. The phenylisopropylhydrazine derivative (JB-516) has also been investigated for its cardiovascular effects both in the dog and in man. In the dog, a dose of 50 mg./kg. will produce blood pressure rise of one hour's duration. Elsewhere in this monograph Sjoerdsma and his colleagues report that in a series of 2 hypertensive patients, JB-516 afforded a smooth lowering of blood pressure of the orthostatic type at a daily dosage of 25 mg.

Mechanism of the blood pressure depression of JB-516. Hornykiewicz[59] has recently shown that iproniazid markedly enhanced blood pressure depression produced by dopamine in the guinea pig. It is conceivable that JB-516 might work via the same mechanism in producing a hypotensive effect in man.

A second possibility is that 2-phenylisopropylhydrazine may have a greater affinity for the cell receptor sites of the blood pressure-regulating centers than norepinephrine and thus block the pressor effects of the endogenous amine. Zeller[60] has postulated an adrenergic blocking effect for iproniazid because of the similarity in "fit" of the hydrazide and epinephrine.

Mechanism of the central excitatory action of the MAO inhibitors. While the MAO inhibitors afford an increase in the brain levels of a variety of endogenous monoamines, their central stimulant effects appear to be due, at least in part to the action of dopamine. The experiments of Carlsson[61] and of Udenfriend[62] show that, after reserpine has been administered to animals, neither iproniazid nor iproniazid plus 5-hydroxytryptophane (the precursor of serotonin that passes the blood-brain barrier) can reverse the sedative effect of this alkaloid.



Active MAO inhibitor

FIGURE 15. Mechanism of action of iproniazid.

However, when iproniazid is administered together with DOPA (the precursor of dopamine that also passes the blood-brain barrier) the animals are aroused from their reserpine-induced stupor. Hence, the dominant action of the MAO inhibitor appears to be mediated by dopamine and norepinephrine rather than by serotonin.

Reduction of the peripheral effects of MAO inhibitors. An ideal central stimulant would be a drug that does not exert its effect until it reaches the brain. Attempts are now being made in our laboratory and elsewhere[62] to combine an active MAO inhibitor with a carrier (for example, an acyl group) that will produce a peripherally inactive enzyme inhibitor. On reaching the brain, this compound would then be cleaved into the active moiety and the acid carrier.

Pletscher has recently shown that the MAO inhibitory activity of iproniazid may be due to its cleavage product: that is, to isopropylhydrazine. This investigator postulates that this cleavage occurs in the brain (FIGURE 15), and that the isonicotinyl group merely acts as a carrier to transport the hydrazine to the brain.

Other acyl groups such as l-glutamyl[63] appear to be more efficient in this respect, since it is claimed that they produce a much more pronounced MAO inhibitory effect in the brain than in other parts of the body. The palmityl derivative, on the other hand, shows a preponderant enzyme inhibitory action on the heart.[63]

Conclusions

The replacement of an amino group by a hydrazine moiety in a series of sympathomimetic amines has yielded a number of potent central stimulants that produce their effect by a dual mechanism: (1) direct stimulation of the CNS (amphetaminelike), and (2) potent MAO inhibition in the brain, which prevents the metabolic destruction of the endogenous central excitatory amines (norepinephrine and dopamine). According to Carlsson and Udenfriend, serotonin is not involved in direct central stimulation.

The structural requirement for optimum central stimulatory properties of the sympathomimetic hydrazines appears to be a 1-phenyl-2-propyl skeleton. Thus, these compounds resemble the amphetamine series with regard to their structural specificity. They differ, however, from the sympathomimetic amines in that (1) they do not produce tachyphylaxis, (2) they elicit hypotensive rather than hypertensive effects in man, (3) they have an extremely prolonged duration of action, (4) they are potent inhibitors of monoamine oxidase in the brain (compared to the relatively weak activities of amphetamine or ephedrine), (5) they do not produce a depression after the stimulant effects have worn off, and (6) they increase appetite instead of decreasing it.

Some rather definite structure-activity relationships have been established that show that the central stimulatory properties of the sympathomimetic hydrazines are dependent upon: (1) The nature of the aromatic ring, (2) The nature of the nuclear substituent, (3) The length and character of the alkyl side chain, and (4) The type and location of the nitrogen substituents.

Future research will be concerned with the development of compounds having a greater tissue specificity.

References

1. Brodie, B. B. & P. A. Shore. 1957. Ann. N. Y. Acad. Sci. **66(3):** 631.
2. Woolley, D. W. & E. Shaw. 1954. Proc. Natl. Acad. Sci. **40:** 228.
3. Gaddum, J. H. 1953. J. Physiol. **121:** 15.
4. Mills, J., R. C. Rathbun & I. H. Slater. 1957. 132nd Meeting, Abstracts of Papers, Am. Chem. Soc. : 6–10.
5. Goodman, L. S. & A. Gilman. 1955. The Pharmacological Basis of Therapeutics. 2nd ed. : 324–328. MacMillan. New York, N. Y.
6. Tripod, J., E. Sury & K. Hoffmann. 1954. Experientia. **10:** 261–262.
7. Brown, B. B. & H. W. Werner. 1954. J. Pharmacol. Exptl. Therap. **110:** 180.
8. Meier, R., F. Gross & J. Tripod. 1954. Klin. Wochschr. **32:** 445.
9. Thomä, O. & H. Wick. 1954. Arch. exptl. Pathol. Pharmakol. Naunyn-Schmiedeberg's. **222:** 540.
10. O'Dell, T. B. & J. M. Mirsky. 1958. Federation Proc. **17**(1): 400.
11. Azima, H. & R. H. Vispo. 1958. Am. J. Psychiat. **115:** 245.
12. Kline, N. R. 1958. San Francisco Meeting, Am. Psychiat. Assoc.
13. Abood, L. G., A. M. Ostfeld & J. H. Biel. 1958. Proc. Soc. Exptl. Biol. Med. **97:** 483.
14. Ostfeld, A. M., L. G. Abood & D. A. Marcus. 1958. A.M.A. Arch. Neurol. Psychiat. **79:** 317.
15. Jacobsen, E. 1957. Antibiotic Med. Clin. Therapy. **5:** 89.
16. Council Report. 1958. J. Am. Med. Assoc, **167:** 1326.
17. Schallek, W. & T. H. F. Smith. 1952. J. Pharmacol. Exptl. Therap. **104:** 291.
18. Goodman, L. S. & A. Gilman. 1955. The Pharmacological Basis of Therapeutics. 2nd ed. : 361. MacMillan. New York, N. Y.
19. Pfeiffer, C. C., E. H. Jenney, W. Gallagher, R. P. Smith, W. Bevan, K. F. Killam, E. K. Killam & W. Blackmore. 1957. Science. **126:** 610.

20. Lemere, F. & J. H. LaSater. 1958. Am. J. Psychiat. **114:** 655.
21. Koelle, G. B. 1958. New Engl. J. Med. **258:** 25.
22. Stoll, W. A. 1947. Schweiz. Arch. Neurol. Psychiat. **60:** 279.
23. Rothlin, E. 1957. Ann. N. Y. Acad. Sci. **66**(3): 668.
24. Benington, F., R. D. Morin & C. C. Clark. 1957. J. Org. Chem. **22:** 232.
25. Brodie, B. B. 1958. Modern Med. (Minneapolis). **26**(16): 69.
26. Selikoff, I. J., E. H. Robitzek & G. G. Ornstein. 1952. Quart. Bull. Sea View Hosp. **XIII:** 17.
27. Loomer, H. P., J. C. Saunders & M. S. Kline. 1957. Paper read at the regional meeting of the Am. Psychiat. Assoc. Syracuse, N. Y.
28. Zeller, E. A., J. Barsky, J. R. Fouts, F. A. Kirshheimer & L. S. Van Orden. 1952. Experientia. **8:** 349.
29. Griesemer, E. C., J. Barsky, C. A. Dragstedt, J. A. Wells & E. A. Zeller. 1953. Proc. Soc. Exptl. Biol. Med. **84:** 699.
30. Rebhun, J., S. M. Feinberg & E. A. Zeller. 1954. *Ibid.* **87:** 218.
31. Biel, J. H., E. G. Schwarz, E. P. Sprengeler, H. A. Leiser & H. L. Friedman. 1954. J. Am. Chem. Soc. **76:** 3149.
32. Beyer, K. H. 1941. J. Pharmacol. Exptl. Therap. **71:** 349.
33. Beyer, K. H. 1946. Physiol. Revs. **26:** 169.
34. Schayer, R. W., R. L. Smiley, K. J. Davis & Y. Kobayashi. 1955. Am. J. Physiol. **182:** 285.
35. Axelrod, J. 1958. Science. **127:** 754.
36. Resnick, O., J. M. Wolfe, H. Freeman & F. Elmadjian. 1958. *Ibid.* : 1116.
37. Craver, B. N., W. Barrett, A. Cameron & F. F. Yonkman. 1951. J. Am. Pharmacol. Assoc. **11:** 559.
38. Schroeder, H. A. 1952. Circulation. **V:** 28.
39. Biel, J. H. 1958. U. S. Patent 2,830,050.
40. Barger, G. & H. H. Dale. 1910. J. Physiol. **41:** 19.
41. Benoit, G. & D. Bovet. 1942. Compt. rend. soc. biol. **136:** 356.
42. Pletscher, A., P. A. Shore & B. B. Brodie. 1955. Science. **122:** 374.
43. Shore, P. A., A. Pletscher & B. B. Brodie. 1956. J. Pharmacol. Exptl. Therap. **116:** 51.
44. Carlsson, A. & N. Hillarp. 1956. Kumgl. Fysiogr. Sallsk. Lund. Forh. **26:** 1.
45. Chessin, M., E. R. Kramer & C. C. Scott. 1957. J. Pharmacol. Exptl. Therap. **119:** 453.
46. Horita, A. 1958. J. Pharmacol. Exptl. Therap. **122:** 176.
47. Maxwell, R. A., A. J. Plummer, J. J. Paytas, S. D. Ross & A. I. Daniel. 1957. Proc. Soc. Exptl. Biol. Med. **94:** 433.
48. Spector, S., D. Prockop, P. A. Shore & B. B. Brodie. 1958. Science. **127:** 704.
49. Biel, J. H., A. E. Drukker, P. A. Shore, S. Spector & B. B. Brodie. 1958. J. Am. Chem. Soc. **80:** 1519.
50. Goodman, L. S. & A. Gilman. 1955. The Pharmacological Basis of Therapeutics. 2nd ed. : 505–537. MacMillan. New York, N. Y.
51. Marsh, D. A. & D. A. Herring. 1950. J. Pharmacol. Exptl. Therap. **100:** 298.
52. Warren, M. R. & H. W. Werner. 1945. *Ibid.* **85:** 119.
53. Hartung, W. 1945. Ind. Eng. Chem. **37:** 126.
54. Spector, S., P. A. Shore & B. B. Brodie. 1958. Fall Meeting Am. Soc. Pharmacol. Exptl. Therap. Ann Arbor, Mich.
55. Walter, L. A., W. H. Hunt & R. J. Fosbinder. 1941. J. Am. Chem. Soc. **63:** 2771.
56. Fromherz, K. & H. Spiegelberg. 1948. Helv. Physiol. Acta. **6:** 42.
57. Barger, G. & A. P. T. Easson. 1938. J. Chem. Soc. **1938:** 2100.
58. Campaigne, E. & W. C. McCarthy. 1954. J. Am. Chem. Soc. **76:** 4466.
59. Hornykiewicz, O. 1958. Brit. J. Pharmacol. **13:** 91.
60. Zeller, E. A. 1958. J. Clin. Exptl. Psychopathol. Quart. Rev. Psychiat. Neurol. **XIX:** 32.
61. Carlsson, A., M. Lindquist & T. Magnusson. 1957. Nature. **180:** 1200.
62. Bogdanski, D. F., H. Weissbach & S. Udenfriend. 1958. J. Pharmacol. Exptl. Therap. **122:** 182.
63. Pletscher, A. 1958. Am. Psychiat. Assoc. Meeting, San Francisco, Calif.

Discussion of the Paper

A. Horita (*University of Washington School of Medicine, Seattle, Wash.*): The presentation of Biel and his colleagues has given some clear indications of the structure-activity relationship of the arylalkylhydrazines as monoamine oxidase inhibitors *in vitro*.

In the intact rat there exists, not only a structure-activity relationship of potency, but also a relationship between chemical structure and monoamine oxidase inhibition of various organs. In our own studies we have determined the liver and brain enzymes after pretreatment with various arylalkyhydrazines and have found that alterations in molecular structure lead to marked changes in their abilities to block monoamine oxidase of these two organs. Thus, two compounds may possess similar *in vitro* potencies but, upon administration of one compound to intact animals, we find that the liver is markedly blocked, while the brain is not; the other compound produces good brain inhibition but little effect on the liver.

Chemical structure therefore plays an extremely important part, not only in potency, but also in the drug's ability to show this type of organ selectivity.

IN VITRO AND *IN VIVO* INHIBITION OF AMINE OXIDASES

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Historical

In view of the widespread interest in the action of iproniazid (Marsilid*) and related hydrazine derivatives on the enzyme, monoamine oxidase (MAO), it is desirable to review briefly the early phases of this line of investigation.

In May 1952 we first described the strong inhibitory effect of iproniazid (Marsilid) on MAO, and the marked difference between the activities of isoniazid and iproniazid.[1] Within the same year the brain and liver MAO of 6 species was blocked both *in vitro* and *in vivo*.[2-4] By the end of 1954 we had recognized that the alkylhydrazine residue of iproniazid is responsible for the MAO-blocking efficiency of the drug, and we had discovered several compounds, including simple alkylhydrazines and benzylhydrazine,[4-11] which, compared with Marsilid, displayed much higher inhibitory power on MAO.

Cooperative efforts between the Departments of Pharmacology, Medicine, and Biochemistry of Northwestern University led to the description of the potentiation of the effect of tyramine and other sympathicomimetic amines on the nictitating membrane of the cat and on the body temperature of the guinea pig.[13, 14] These and other results clearly demonstrated that iproniazid, by blocking MAO, interfered with the amine metabolism of animals. They also furnished a basis for explaining certain side reactions encountered in the therapeutic application of the drug, which later attracted much clinical interest. In 1953 Schayer, then associated with Northwestern University, was able to protect labeled tyramine with iproniazid against metabolic degradation.[15] Other contributions during this early period were made in 1953 by Viollier *et al.* at the University of Basel, Basel, Switzerland,[16] and by Koelle and his colleagues at the University of Pennsylvania, Philadelphia, Pa.[17] Koelle used iproniazid in his work on the histochemical localization of MAO. The iproniazid tool was ready for use when the avalanche of serotonin investigations descended. Papers by Sjoerdsma *et al.*[18] in 1955 and by Chessin *et al.* early in 1956[19] heralded the influence that iproniazid was to exercise on the study of the metabolism and physiology of serotonin. Since then iproniazid, which had been regarded solely as an antituberculous agent, has been restored to circulation in a new capacity.

Many papers published during the past two years added substantially to the previously developed concepts. However, we are not satisfied with this situation because we are well aware of the many gaps in our knowledge. In an attempt to express our attitude we paraphrase George Santayana: we intend to strain our concepts through the utmost rigors of skepticism and to approach every opinion, whatever our own ultimate conclusion, with the courtesy and smile of the skeptic.

---

* Hoffmann-La Roche.

Nomenclature and Classification of Amine Oxidases

To simplify future discussions we offer the following on nomenclature and classification of amine oxidases (TABLE 1).

The term amine oxidases logically covers the whole group of enzymes rather than any subdivision of it. The enzymes attacking aliphatic compounds with terminal amino groups can be separated operationally by 1 mM semicarbazide.[20] In this definition, semicarbazide cannot be replaced by the term carbonyl reagent, since many carbonyl reagents such as monosubstituted alkyl and aryl hydrazines act on both enzymes efficiently, while only monosubstituted acyl hydrazines exclusively inhibit the group of diamine oxidases. The monoamine oxidases display only one kind of receptor in their active center, while diamine oxidases possess two kinds.

Objections to the Iproniazid-MAO Concept

Much remains to be learned about the role of MAO in the biological action of iproniazid and related compounds. However, some of the data advanced against the concept of MAO being involved in the iproniazid mechanism are open to criticism. As an illustration, three examples are discussed:

TABLE 1
CLASSIFICATION OF AMINE OXIDASES

(1) Nonterminal amines
    Amphetamine oxidase
(2) Terminal amines
    (a) Semicarbazide-resistant monoamine oxidases with 1 type of receptor
    (b) Semicarbazide-sensitive diamine oxidases with 2 types of receptors

(1) Referring to the work of Zatman *et al.*,[21, 22] it is assumed[23, 24] that iproniazid may interfere with the diphosphopyridine nucleotide metabolism. As a matter of fact, iproniazid displays a weak inhibitory power on diphosphopyridine nucleotidase (DPNase). The pI_{50} values* for isoniazid (INH) and iproniazid are 4.0 and 2.7, respectively. Therefore, isoniazid is 20 times more effective than iproniazid, while in most physiological systems isoniazid is almost inactive, in accordance with its inability to block MAO. Only the ruminants and birds tested appear to have INH-sensitive DPNases, whereas the remaining species examined, including man and laboratory mammals, are apparently insensitive. Iproniazid concentrations up to M/50 are without any influence. For most iproniazid-sensitive preparations, such as sheep spleen DPNase, the concentration required for the production of substantial inhibitions is so high that they can hardly be achieved *in vivo*. Finally, no single connection between the enzymic effect and the biological action of iproniazid has been brought forth. Surely, DPNase could still play a role; however, new evidence is required to reopen this question.

(2) Another alternative to the iproniazid-MAO concept is easily dismissible. It has been considered that iproniazid may influence the animal organism by depleting it of pyridoxine.[23, 24] As evidence, a paper by Biehl and Vilter[25] is

* Fifty per cent potency inhibition.

cited. However, iproniazid is not mentioned by these authors, whose study pertains to isoniazid alone. As a carbonyl reagent, isoniazid can form a hydrazone with pyridoxal. We know of no *in vitro*[26] experiments the results of which suggest a direct chemical interaction between pyridoxine derivatives and iproniazid.

(3) In many laboratories it has been observed that MAO is blocked *in vivo* by iproniazid, and that various biogenic amines, including serotonin, are protected against oxidative deamination. The conclusion by Udenfriend *et al.*[27] that iproniazid does not reach the site of MAO *in vivo* was therefore puzzling. These authors injected iproniazid into animals from which they afterward prepared liver slices. Serotonin, added to the slices, continued to disappear. However, a different explanation was later found for this phenomenon; in liver slices serotonin is inactivated not only by MAO[10] but also by coupling with glucuronic acid.[28] Brain slices obtained from iproniazid-injected rats did not destroy serotonin; thus, in brain MAO seems to be the predominating serotonin-inactivating agent.

Inactivation of Biogenic Amines by Methylation and by Glucuronide Formation Versus Oxidative Deamination

Catecholamines, as found by Armstrong and McMillan[29] and by Axelrod[30] can be metabolized by methylation of the *meta*-hydroxy group. Since iproniazid increases the urinary excretion of methylated epinephrines, it was concluded that catecholamines are methylated first and deaminated afterward. These results offer clear-cut proof for the ability of the animal metabolism to methylate epinephrine before this compound is deaminated. They also demonstrate the usefulness of iproniazid as a biochemical tool. However, there is no reason to assume that in every instance the catecholamine is going through methylation first. Finally, brain and, in particular, heart tissues seem to be very poor sources for the methylating enzyme O-methyltransferase. It may not be a mere coincidence that potentiation and protection of catecholamines by iproniazid has been reported for these organs.[31, 32] Similar concepts seem to apply to the degradation of serotonin by MAO as compared with the inactivation by formation of glucuronide.

Relationships Between Chemical Structure and Action on Amine Oxidases

Apart from the above discussion of data in the literature, we present a small segment of our recent work. We were interested primarily in the elucidation of the structure of the active center of amine oxidases. Previously we had concluded that Marsilid and other hydrazine derivatives act on the same part of the enzyme surface as do the substrates.[10] Hydrazines were considered to be pseudoamines. In agreement with this concept we found that the optimal chain length of the aliphatic amines as substrates of hog liver MAO is 4, while for the same enzyme propyl hydrazine is the most effective inhibitor in this series. Obviously, the NH-residue seems to be equivalent to one CH_2-group.

We prepared phenylalkylamines and tested their rate of degradation in the presence of MAO. As may be seen from TABLE 2 the affinity is higher for phenylethylamine than for benzylamine, phenylpropylamine, and phenylbutylamine. We then synthesized the corresponding phenyl alkylhydrazines and attempted to block MAO with them. Benzylhydrazine, the isosteric analogue of phenylethylamine, was the best inhibitor, with phenylethylhydrazine a close second. While for hydrazines, with their tremendous affinity for this enzyme, the length of the aliphatic chain is of lesser importance than for the substrates (amines), the effect of increasing the aliphatic chain is quite visible (TABLE 3). The action of phenylpropylhydrazine is markedly weaker than that of the two preceding hydrazine derivatives. Introduction of a double bond in β-γ position (last compound in TABLE 3) reduces still further the inhibitory power of the propyl derivatives.

TABLE 2
AFFINITIES BETWEEN MAO AND PHENYLALKYLAMINES

	$1/k_M$
C_1	350
C_2	430
C_3	290
C_4	300

TABLE 3
pI VALUES FOR MAO AND PHENYLALKYLHYDRAZINES

	pIox
C_0	4.3
C_1	6.0
C_2	5.6
C_3	4.5
C_3, unsat.*	3.5

* Prepared by the Lakeside Laboratories, Inc., Milwaukee, Wis.

TABLE 4
pI VALUES FOR DAO AND PYRIDINE CARBONIC ACID HYDRAZIDES

	pIox
INH	4.0
4-Pyrid. acet.	4.0
4-Pyrid. prop.	5.7
3-Pyrid. acet.	6.0

Earlier we described similar relationships between the length of the aliphatic chain and the optimal blocking efficiency of isoniazid homologues on MAO.[11, 33] Similar examples were found for diamine oxidase (DAO). Increase of the chain length in isoniazid* by 1 unit did not change the pI_{50} value (TABLE 4). However, the inhibitory power was 50 times higher for the propionic acid homologue. Introduction of the 2-membered chain into the 3-position of the pyridine ring produces a one-hundredfold increase. These results are in accordance with our concept that the hydrazines are pseudoamines and that they act on the same part of the enzyme as do the substrates (TABLE 5).

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* The compounds were prepared by Hoffmann-La Roche Inc., Nutley, N. J.

Finally, to exemplify how very small changes in the structure of hydrazine derivatives lead to marked differences in blocking efficiency, we present our data on some benzylhydrazine analogues (TABLE 5). Switching from oxygen to sulfur or from the 2- to the 3-position of the pyridine nucleus produces an increase in pI-values by 2 units, which equal a hundredfold greater efficiency. We suspect that, for the first pair, hydrogen bonding is responsible for this difference.

We are pleased that so few small seeds sown in the field of enzymology have brought forth such a strong tree, pushing its branches in so many directions.

TABLE 5
pI VALUES FOR MAO AND BENZYLHYDRAZINE ANALOGUES*

	pIox	pIam
Furan-2-	4.5	4.6
Thiophene-2-	6.7	6.6
Pyridine-2-	4.7	4.6
Pyridine-3-	6.7	6.6

* Prepared by Lakeside Laboratories, Inc., Milwaukee, Wis.

References

1. ZELLER, E. A., J. BARSKY, J. R. FOUTS, W. F. KIRCHHEIMER & L. S. VAN ORDEN. 1952. Influence of isonicotinic acid hydrazide (INH) and 1-isonicotinyl-2-isopropyl hydrazine (IIH) on bacterial and mammalian enzymes. Experientia. **8:** 349.
2. ZELLER, E. A., J. BARSKY, E. R. BERMAN & J. R. FOUTS. 1952. Action of isonicotinic acid hydrazide and related compounds on enzymes involved in the autonomic nervous system. J. Pharmacol. Exptl. Therap. **106:** 427–428.
3. ZELLER, E. A., J. BARSKY, E. R. BERMAN & J. R. FOUTS. 1952. Action of isonicotinic acid hydrazide and related compounds on enzymes of brain and other tissues. J. Lab. Clin. Med. **40:** 965–966.
4. ZELLER, E. A. & J. BARSKY. 1952. *In vivo* inhibition of liver and brain monoamine oxidase by 1-isonicotinyl-2-isopropylhydrazine. Proc. Soc. Exptl. Biol. Med. **81:** 459–461.
5. BARSKY, J., E. R. BERMAN & E. A. ZELLER. 1953. On the solubilization and iproniazid inhibition of monoamine oxidase. XIX. Intern. Physiol. Congr. Abstr. : 191–192.
6. BARSKY, J., E. R. BERMAN, J. R. FOUTS & E. A. ZELLER. 1954. Effect of antitubercular drugs on the metabolism of histamine and sympathomimetic amines. 10th Ann. Meeting, Am. Acad. Allergy. Abstr. : 15–16.
7. ZELLER, E. A., J. BARSKY & J. FUGGER. 1954. Effect of substituted phenylhydrazines and isonicotinylhydrazines on monoamine oxidase. 126th Meeting Am. Chem. Soc. Abstr. : 46C.
8. ZELLER, E. A. 1955. Allgemeine Physiologie und Pathologie der Enzyme. Handbuch der allgemeinen Pathologie. **2:** (1). Das Zytoplasma. Springer. Berlin, Germany.
9. ZELLER, E. A., J. BARSKY, J. R. FOUTS & J. C. LAZANAS. 1955. Structural requirements for the inhibition of amine oxidases. Biochem. J. **60:** v.
10. ZELLER, E. A., J. BARSKY & E. R. BERMAN. 1955. Amine oxidases. XI. Inhibition of monoamine oxidase by 1-isonicotinyl-2-isopropylhydrazine. J. Biol. Chem. **214:** 267–274.
11. ZELLER, E. A. 1956. The fate of histamine in the body, with particular reference to the enzymology of histamine oxidation. Ciba Foundation Symposium on Histamine. : 339–349. Churchill. London, England.
12. ZELLER, E. A. 1956. On the nature of the reactive surface of amine oxidases. Symposium on Biochemistry and Nutrition, Cornell Univ., Ithaca, N. Y. : 25–33.
13. GRIESEMER, E. C., J. BARSKY, C. A. DRAGSTEDT, J. A. WELLS & E. A. ZELLER. 1953. Potentiating effect of iproniazid on the pharmacological action of sympathomimetic amines. Proc. Soc. Exptl. Biol. Med. **84:** 699–701.
14. REBHUN, J., S. M. FEINBERG & E. A. ZELLER. 1954. Potentiating effect of iproniazid

on action of some sympathicomimetic amines. Proc. Soc. Exptl. Biol. Med. **87:** 218–220.

15. Schayer, R. W. 1953. *In vivo* inhibition of monoamine oxidase studied with radioactive tyramine. Proc. Soc. Exptl. Biol. Med. **84:** 60–63.
16. Viollier, G., E. Quiring & H. Staub. 1953. Einfluss von oral verabreichtem Isonicotinsäurehydrazid und dessen Isopropylderivat auf den Enzymhaushalt der weissen Ratte. Helv. Chim. Acta **36:** 724–730.
17. Koelle, G. B. & A. de T. Valk. 1954. Physiological implications of the histochemical localization of monoamine oxidase. J. Physiol. **126:** 434–447.
18. Sjoerdsma, A., T. E. Smith, T. D. Stevenson & S. Udenfriend. 1955. Metabolism of 5-hydroxytryptamine (serotonin) by monoamine oxidase. Proc. Soc. Exptl. Biol. Med. **89:** 36–38.
19. Chessin, M., B. Dubnick, E. R. Kramer & C. C. Scott. 1956. Modifications of pharmacology of reserpine and serotonin by iproniazid. Federation Proc. **15:** 409.
20. Zeller, E. A. 1959. The role of amine oxidases in the destruction of catecholamines. Pharmacol. Rev. In press.
21. Zatman, L. J., N. O. Kaplan, S. P. Colowick & M. M. Ciotti. 1954. Effect of isonicotinic acid hydrazide on diphosphopyridine nucleotidases. J. Biol. Chem. **209:** 453–466.
22. Zatman, L. J., N. O. Kaplan, S. P. Colowick & M. M. Ciotti. 1954. The isolation and properties of the isonicotinic acid hydrazide analogue of diphosphopyridine nucleotide. J. Biol. Chem. **209:** 467–484.
23. Udenfriend, S., H. Weissbach & D. F. Bogdanski. 1957. Biochemical findings relating to the action of serotonin. Ann. N. Y. Acad. Sci. **66**(3): 602–608.
24. Koelle, G. B. 1958. Pharmacologic significance of inhibition of monoamine oxidase. J. Clin. Exptl. Psychopathol. & Quart. Rev. Psychiat. Neurol. **19:** Suppl. 1, 37–44.
25. Biehl, J. P. & R. W. Vilter. 1954. Effect of isoniazid on vitamin B_6 metabolism; its possible significance on producing isoniazid neuritis. Proc. Soc. Exptl. Biol. Med. **85:** 389–392.
26. Carbon, J. A., W. P. Burkard & E. A. Zeller. 1958. Über die Wirkung von symmetrischen 1,2-Dialkylhydrazinen auf Carbonylverbindungen und Amin-oxydasen. Helv. Chim. Acta. **41:** 1883–1889.
27. Udenfriend, S., H. Weissbach & D. F. Bogdanski. 1957. Effect of iproniazid on serotonin metabolism *in vivo*. J. Pharmacol. Exptl. Therap. **120:** 255–260.
28. Weissbach, H., B. G. Redfield & S. Udenfriend. 1958. Serotonin-O-glucuronide; an alternate route of serotonin metabolism. Federation Proc. **17:** 418.
29. Armstrong, M. D. & A. McMillan. 1957. Identification of a major urinary metabolite of norepinephrine. Federation Proc. **16:** 146.
30. Axelrod, J. 1958. Presence, formation, and metabolism of normetanephrine in the brain. Science. **127:** 754–755.
31. Shore, P. A., J. A. R. Mead, R. G. Kuntzman, S. Spector & B. B. Brodie. 1957. On the physiologic significance of monoamine oxidase in brain. Science. **126:** 1063–1064.
32. Pletscher, A. 1958. Einfluss von Isopropyl-isonicotinsäurehydrazid auf den Catecholamingehalt des Myocards. Experientia. **14:** 73.
33. Barsky, J., W. L. Pacha, S. Sarkar & E. A. Zeller. 1959. Amine oxidases. XVII. Mode of action of 1-isonicotinyl-2-isopropylhydrazine on monoamine oxidase. J. Biol. Chem. **234:** 389–391.

Discussion of the Paper

Sidney Udenfriend (*Laboratory of Clinical Biochemistry, National Heart Institute, Public Health Service, Bethesda, Md.*): I propose to make three points that stem from previous papers, as well as from that of Zeller.

First is the suggestion that we refrain from relating these observed effects solely to norepinephrine and serotonin. If one of these potent amine oxidase inhibitors is administered, it is apparent from studies in our laboratory, and I believe in other laboratories, that a liberation of all kinds of amines is induced, including some that have not been known to occur normally. Decarboxylation of tryptophan to tryptamine apparently occurs normally, but it has been detected only through the use of these agents. Tyramine, which has been considered a foreign compound and studied as a pharmacological tool, is normally found in urine. It increases markedly when monoamine oxidase (MAO) inhibitors are administered. Other amines also have been detected in urine after administering MAO inhibitors. I am sure that these and other amines are normally formed, but we have not had available methods to find them, although they appear to be present in amounts equivalent to serotonin or norepinephrine. MAO inhibitors thus provide us with a tool for investigating new pathways of amine metabolism. In view of these findings I think that we should use caution in ascribing the effects of MAO inhibition to any particular amine.

The second point is the relationship between clinical effects and MAO inhibition. Attempts have been made to relate physiological effects to MAO inhibition in animals. However, in man, where a drug is administered in amounts that are not harmful, there is some question as to whether these compounds are really acting as MAO inhibitors. It is necessary to use caution also in equating the effects of a given drug in man; concerning a particular enzymatic effect, we must use procedures appropriate to determine whether the effect occurs in the patient during therapy. Elsewhere in this monograph my colleague Albert Sjoerdsma discusses the relationships between dosage and MAO inhibition in man.

Third, the discussions on the relationship of chemical structure to monoamine oxidase inhibition must be considered from various aspects. If pure enzymes are used, a certain type of relationship is observed. By using mitochondria, whole cells, tissue slices and, of course, the whole animal, the complexity of the system is increased. I am sure that, depending upon the system used, one will obtain different relationships between chemical structure and MAO inhibition. Furthermore, differences are found between homogenates prepared from brain and those from liver or when homogenates of the same tissue prepared from two different species are compared. Studies carried out on crude homogenates cannot be used as an absolute measure of enzyme inhibition. Results of comparisons from laboratory to laboratory may agree only as to order of magnitude. As for the theoretical aspect of drug-enzyme interaction, this can be ascertained only at the level of the pure enzyme. Of course, some drugs that are most active at the level of the pure enzyme may be least active when administered *in vivo*.

The final point is that, whereas most of the studies reported here concern hydrazines, these are not the most potent amine oxidase inhibitors; they merely have been the most actively investigated. One can find agents that, in *in vitro* tests, are as potent as the most potent hydrazines. The *harmala* alkaloids represent a whole series of such potent nonhydrazine MAO inhibitors, and there are others as well. What may be considered as a mechanism of MAO inhibition in work with hydrazines may be totally irrelevant when considered from the standpoint of other inhibitors. All of these points should be kept in mind.

PHARMACOLOGY OF JB-516 (PIH)

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The possible relationship between monoamine oxidase (MAO) inhibition and central nervous system actions of drugs has led to the search for newer and more potent inhibitors. The compound β-phenylisopropylhydrazine (JB-516 or PIH; see FIGURE 1) was recently reported to possess marked anti-MAO activity both *in vitro* and *in vivo*.[1] Its ability to raise the brain levels of serotonin (5-hydroxytryptamine) and norepinephrine in experimental animals has also been described.[2, 3] Possessing a structure very similar to that of the central nervous system stimulant amphetamine, PIH exhibits many of the pharmacological actions of the amine. For instance, it produces a typical amphetaminelike pressor response with doses ranging from 0.05 to 5.0 mg./kg. in the anesthetized dog. Repeated administration of the drug leads to tachyphylaxis, after which amphetamine no longer exerts its usual pressor response. In animals pretreated with amphetamine PIH also exhibits no pressor response, indicating a cross-tachyphylaxis between the two drugs. PIH also possesses some of the direct central nervous system stimulant action, although it is quite weak when compared with amphetamine. The hydrazine structure also appears to give it some of the properties found in iproniazid, such as the potentiation of the hexobarbital sleeping time. These and other actions of PIH make it an extremely interesting compound; its MAO-inhibiting action, however, is by far the most significant of its properties. It is the purpose of this paper to present some of the results we have observed on the MAO-inhibiting property of PIH.

MAO Inhibition by PIH

MAO activity of rat liver and brain homogenates were determined according to the method described by Udenfriend *et al.*,[4] using serotonin as the substrate. After incubation of the substrate with the enzyme preparation for 15, 30, or 60 min., the amount of serotonin remaining was extracted, reacted with the nitrosonaphthol reagent, and measured spectrophotometrically. All data are expressed as percentages of serotonin metabolized. In the *in vitro* experiments PIH concentrations of between 10^{-5} M and 10^{-7} M were used. It was found that, at 10^{-6} M, PIH was quite effective in inhibiting MAO in both brain and liver homogenates (FIGURE 2). Concentrations above 5×10^{-6} M were found to produce complete inhibition, while at 10^{-7} M little or no inhibition was seen. Compared with iproniazid, PIH at 10^{-6} M produced approximately the same degree of inhibition as iproniazid at 5×10^{-5} M. PIH was also found to be an effective MAO inhibitor *in vivo*. Doses of 10 to 20 mg./kg. were effective in completely inhibiting rat liver MAO. When the duration of the inhibitory effect was determined, PIH, like iproniazid, exhibited an extremely prolonged effect. Even a week after a single dose of PIH, MAO inhibition was still demonstrable. As may be seen in FIGURE 3, there is a gradual recovery of MAO activity over the 1-week period, at which time about 80 per cent of the activity had returned.

Potentiation of the 5-HTP Pyretogenic Effects

PIH was also employed in our studies on the pyretogenic effect of 5-hydroxytryptophan (5-HTP). In doses of 50 mg./kg. or more, 5-HTP produces hyperthermia similar to that seen with lysergic acid diethylamide (LSD-25).[5, 6] This hyperthermia was greatly potentiated by iproniazid, indicating that MAO

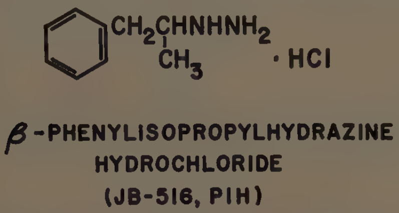

FIGURE 1. Chemical structure of β-phenylisopropylhydrazine hydrochloride (Lakeside Compound No. JB-516, PIH).

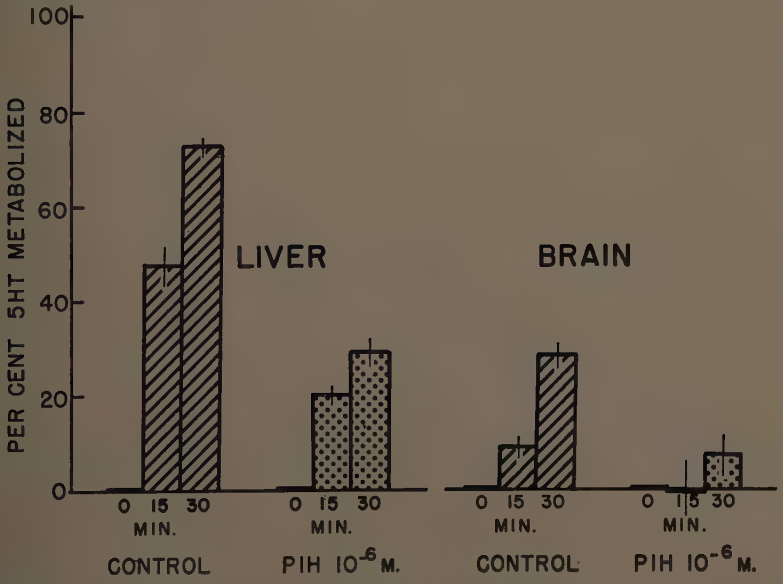

FIGURE 2. The monoamine oxidase inhibition of rat liver and brain homogenates by PIH at the concentration of 10^{-6} M. Each bar represents the mean of 5 determinations. Vertical lines represent the standard error of the means.

blockade led to increased levels of 5-HT, which was being biosynthesized from 5-HTP. When PIH in a dose of 5 mg./kg. was given to normal rabbits, no rectal temperature changes were noted. If, however, this dose was followed by 10 mg./kg. of 5-HTP, a marked hyperthermia and excitation of the rabbits were observed. In all cases the temperatures rose rapidly to above 43° to 44°C., and death resulted at the peak of the temperature response (FIGURE 4). The dose of 5-HTP used in these experiments was not hyperthermic in control rabbits. When 5-HTP was decreased to 5 mg./kg. and administered to PIH-treated rabbits, hyperthermia of a smaller degree was produced, and all animals

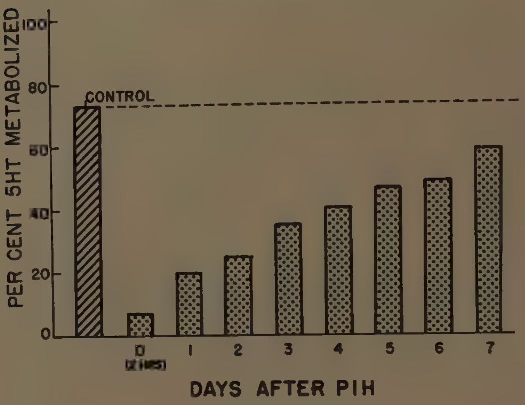

FIGURE 3. The duration of monoamine oxidase inhibition produced in rat liver after a single administration of a dose of 20 mg./kg. of PIH. Livers were removed from these rats at the designated times and assayed as in the *in vitro* determinations. Each bar, with the exception of the control, represents the mean of the liver monoamine oxidase activities from 2 rats.

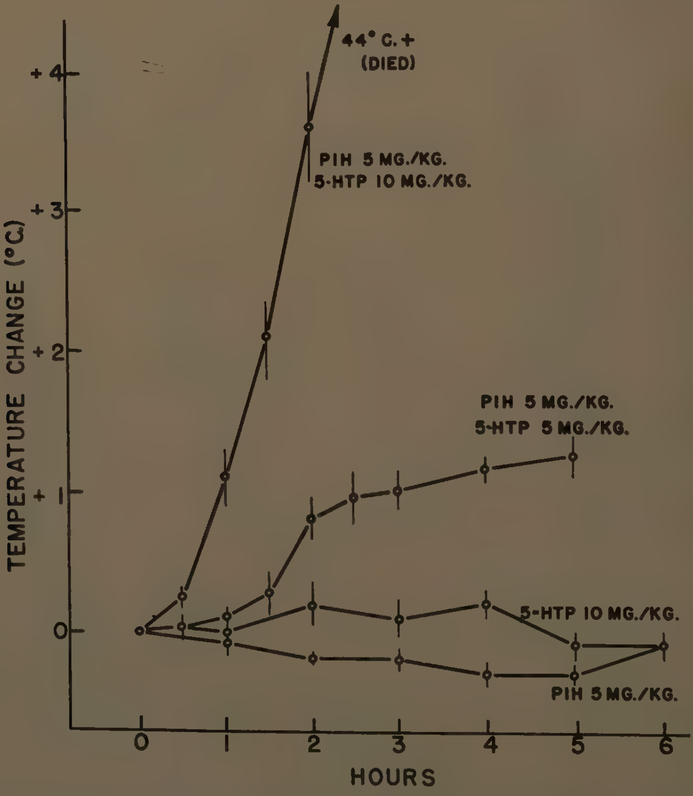

FIGURE 4. The potentiation of the 5-hydroxytryptophan pyretogenic effect of PIH. Each curve represents the mean temperature changes of 4 rabbits after the administration of the designated drugs. The vertical lines represent the standard errors of the means.

recovered within 5 to 8 hours. As with the MAO inhibition, the ability of PIH to potentiate the 5-HTP pyretogenic effect was also found to be of a prolonged nature. Rabbits pretreated with 10 mg./kg. of PIH showed marked potentiation to subpyretogenic doses of 5-HTP for at least 6 days thereafter. Nine days after the original PIH administration there was still some noticeable potentiating effect present.

These studies indicate that the potentiation of the 5-HTP pyretogenic effect is a function of the MAO-inhibitory property of PIH. Similar results have been observed when either reserpine or bufotenine is administered to PIH-pretreated rabbits, giving further evidence that MAO blockade is responsible for the potentiation.

Comparison of Liver and Brain MAO Effects in Vivo[7]

We next turned to the *in vivo* studies in rats to determine some of the characteristics of the PIH inhibition of MAO. Comparisons with iproniazid were made, since this drug had been most widely investigated by various workers.[8, 9] Our primary interest in these studies was to determine possible differences in organ selectivity for the two compounds. The relative degrees of MAO inhibition in brain and liver at varying dose levels were therefore determined. Doses ranging from 1×10^{-7} moles/kg. to 2.5×10^{-5} moles/kg. of PIH and 1×10^{-6} moles/kg. to 2.5×10^{-4} moles/kg. of iproniazid were used. Both brain and liver from each animal were assayed for MAO activity.

It was interesting to find that PIH and iproniazid were approximately equiactive in inhibiting liver MAO *in vivo*. Inhibition became evident at 2.5×10^{-6} moles/kg. and progressed with increasing dose levels. At 5×10^{-5} moles/kg. both PIH and iproniazid had produced essentially complete liver inhibition. Brain, on the other hand, showed a completely different picture, in that it was inhibited by much smaller doses of PIH than by iproniazid. At 5×10^{-7} moles/kg. of PIH, brain MAO showed decreased activity, while 5×10^{-6} moles/kg. was sufficient to inactivate the enzyme completely. In contrast, iproniazid did not show brain MAO inhibition until the dose level of 2.5×10^{-5} moles/kg., and complete inhibition occurred at 2.5×10^{-4} moles/kg. (see FIGURE 5). It is quite evident from these *in vivo* studies that PIH produces MAO inhibition in brain at much lower doses than iproniazid, while in liver both drugs are approximately equiactive. When the liver and brain MAO activities of animals pretreated with progessively increasing doses of the inhibitors were analyzed simultaneously, the following observations were made:

(1) Iproniazid inhibits liver MAO with much smaller doses than it does the brain. The brain enzyme is affected only after marked liver MAO inhibition is produced. A dose that produces a 50 per cent inhibition of brain MAO is sufficient to block liver MAO completely.

(2) With PIH the brain enzyme is attacked with smaller doses than is liver. Brain MAO is almost completely inactive with doses just effective against liver MAO. These results are shown in FIGURE 5.

Discussion

The work that has been reviewed in this presentation stresses two important facts about PIH. First, PIH is a highly potent MAO inhibitor both *in vitro* and *in vivo*; second, it has a higher selectivity for brain than for liver in the intact animal. The fact that *in vitro* both brain and liver homogenates are equally inhibited by PIH indicates the absence of a greater affinity for the brain enzyme. Rather, it would appear that the different degrees of MAO inhibition in the two tissues are caused primarily by a different distribution of the drugs. PIH seems to reach the brain rapidly and with ease, while iproniazid does so with difficulty and only with relatively larger doses. This great difference between the two drugs is most probably the result of their differences in chemical structure. The poor distribution of iproniazid to the brain, as well as its lower potency (as compared with PIH), may be due in part to the presence of the isonicotinyl moiety in structure. This is supported by the finding of Davison,[10] who showed isopropylhydrazine to be much more potent than iproniazid *in vitro*. We have also studied the compounds isopropylhydrazine and 1-isonicotinyl-2-phenylisopropylhydrazine* for their MAO properties both *in vitro* and *in vivo*.[11] FIGURE 6 shows the structural relationships of PIH, iproniazid, isopropylhydrazine, and JB-821. It may be seen that JB-821 differs from PIH only by the presence of a terminal N-isonicotinyl group and from iproniazid by a phenyl group on the alkyl chain. This compound was found to be a much weaker MAO inhibitor than PIH, although somewhat more potent than iproniazid. Like iproniazid, its ability to block liver MAO *in vivo* was much greater than for brain. In order to demonstrate inhibition of brain enzyme doses, 2 to 5 times as great were needed. Isopropylhydrazine lacks the benzene ring of PIH and the isonicotinyl group of

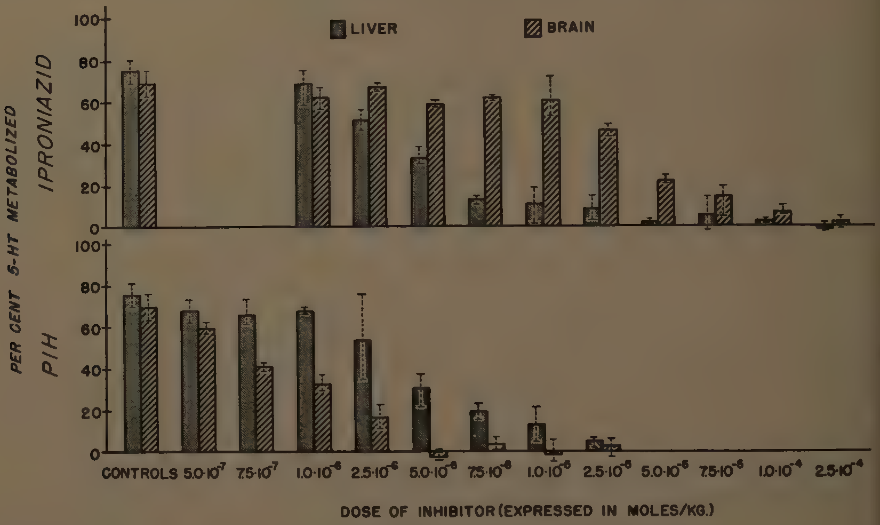

FIGURE 5. The relative activities of liver and brain monoamine oxidase from rats pretreated with varying doses of either iproniazid or PIH. Tissues were removed from the animals 4 hours after administration of the inhibitors. Each bar in the graph represents the mean of 3 animals. The dotted vertical lines indicate the range obtained for each dose.

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* JB-821, Lakeside Laboratories, Inc., Milwaukee, Wis.

iproniazid. It was found to be an extremely potent MAO inhibitor but, like iproniazid and JB-821, it also exhibited much greater MAO blockade against liver than against brain *in vivo*. From these results it is reasonable to assume that in the case of iproniazid its relative inability to inhibit brain MAO *in vivo* may be a function of both the isopropylhydrazine and the isonicotinyl moieties of the molecule. In JB-821 we may also assume that only the isonicotinyl portion hinders its entrance into brain. Finally, the selectivity of PIH for brain MAO *in vivo* may be attributed to the presence of the benzene ring in its structure, since isopropylhydrazine does not possess this characteristic.

It is evident from these studies that, in the search for newer MAO inhibitors that might be useful in central nervous system depression, it is necessary to consider not only the potency of the drug but also its ability to reach the brain in sufficient quantities. Since PIH possesses both of these qualities, it should be possible to produce the desired central nervous system effects by doses lower than those required to produce side effects or toxic effects that might arise through excessive accumulation of the drug in the liver and other peripheral tissues.

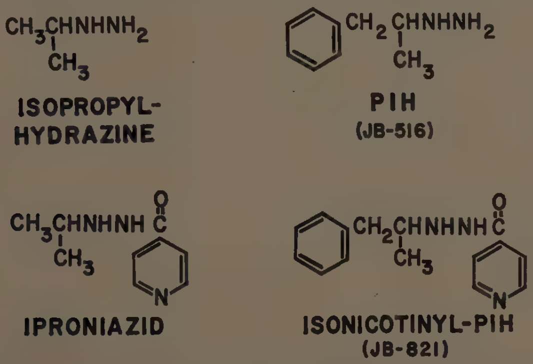

FIGURE 6. The chemical structures of the compounds PIH, iproniazid, *l*-isonicotinyl 2-phenylisopropylhydrazine (JB-821), and isopropylhydrazine.

References

1. Horita, A. 1958. J. Pharmacol. Exptl. Therap. **122:** 176–181.
2. Shore, P. A. & B. B. Brodie. 1958. Science. **127:** 704.
3. Brodie, B. B., S. Spector, R. G. Kuntzman & P. A. Shore. 1958. Naturwiss. **10:** 243–244.
4. Udenfriend, S., H. Weissbach & B. B. Brodie. 1958. Methods of Biochemical Analysis. **6:** 95–130. Interscience. New York, N. Y.
5. Horita, A. 1954. Science. **120:** 1100–1101.
6. Horita, A. & J. H. Gogerty. 1958. J. Pharmacol. Exptl. Therap. **122:** 195–200.
7. Gogerty, J. H. & A. Horita. 1958. In preparation.
8. Zeller, E. A., J. Barsky & E. R. Berman. 1955. J. Biol. Chem. **214:** 267–274.
9. Schayer, R. W. 1953. Proc. Soc. Exptl. Biol. Med. **84:** 60–63.
10. Davison, A. N. 1957. Biochem. J. **67:** 316–322.
11. Horita, A. & R. G. Parker. 1958. In preparation.

Discussion of the Paper

Harris L. Friedman (*Lakeside Laboratories, Inc., Milwaukee, Wis.*): I have only a few brief comments to make. I think Horita's data are clear and that his paper speaks for itself. Of course, the question as to what occurs in humans or in animals when the same drug is given by a different route of administration remains unanswered.

Concerning structure-activity relationships, I think it is a wise man who prepares and tests only three or five compounds, for then he can write a wonderful paper on structure-activity correlations. The more compounds one tests, the more the exceptions one finds, since differences in processes such as absorption, excretion, and metabolism occur. However, these are exactly the unknowns that make life interesting for the organic chemist who is designing drugs. It is not that he ignores them; it is simply that he does not know of these factors until some compounds have been made, screened, and adequately tested. When he gets this information, he attempts to utilize it in making future compounds.

As I read various biochemical studies discussed by the contributors to this monograph, I thought about the tremendous difference in outlook today in what is considered the proper work-up for a new drug as compared to the situation about twenty years ago. I think that much of the impetus in investigating problems of absorption, metabolism, and excretion is due to Brodie and his co-workers, who have studied mechanisms that pertain to the actions of drugs and have focused attention on aspects that must be considered today, but which were completely ignored in the past.

BIOCHEMICAL AND PHARMACOLOGICAL STUDIES OF β-PHENYLETHYLHYDRAZINE AND SELECTED RELATED COMPOUNDS

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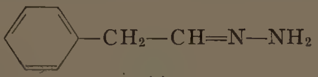
Warner-Lambert Research Institute, Morris Plains, N. J.

Introduction

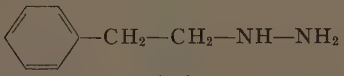
Our initial interest in compounds capable of inhibiting monoamine oxidase (MAO) was prompted by the observations of Brodie and his associates that rabbits pretreated with the MAO inhibitor iproniazid and then injected with reserpine were not sedated, but displayed signs of stimulation.[1]

Many hydrazines and hydrazides that had been synthesized for antitubercular activity in 1952 were re-examined as possible MAO inhibitors. Special consideration was given to hydrazine analogues of phenylethylamine because of their close chemical relationship to many of the biogenic amines.

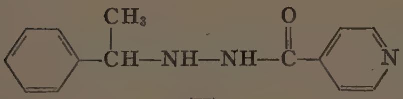
The structural relationship of some of the more interesting compounds is shown below.



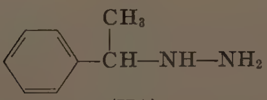
(I)



(IA)



(II)



(IIA)

Compound I, phenylethylidenehydrazine, proved to be inactive as an inhibitor of monoamine oxidase. However, simple reduction of this compound yielded a rapid-acting potent inhibitor with long duration of action, namely, compound IA, β-phenylethylhydrazine (W-1544). Subsequent studies with compound II, an isonicotinyl derivative of α-phenylethylhydrazine, revealed that it is a potent MAO inhibitor. Removal of the isonicotinyl portion of the molecule resulted in compound IIA, α-phenylethylhydrazine, a compound with increased potency over both compounds IA and II.

Other structurally related compounds were subsequently prepared and screened in mice by the "reserpine challenge test,"[2, 3] as well as by biochemical procedures. Some of the biochemical and pharmacological data obtained with β-phenylethylhydrazine and certain related compounds are reported in this paper.

Methods

All dosages reported in this paper are in terms of base.

Serotonin was extracted in butanol[4] and assayed spectrophotofluorometrically.[5] All determinations were run in duplicate on 2 pooled mouse brains.

Monoamine oxidase activity was measured manometrically. Oxygen uptake was followed with a conventional Warburg apparatus, using 0.01 M tyramine hydrochloride as substrate in 0.06 M sodium phosphate, *p*H 7.1, in the presence of 0.001 M sodium cyanide. Dialyzed homogenates of 3 pooled whole mouse brains served as the enzyme source. For correction, endogenous oxygen uptake was measured by addition of substrate to homogenate previously inhibited *in vitro* by preincubation with a high concentration of β-phenylethylhydrazine (0.005 M).

Acute toxicity studies were conducted on fasted female albino mice weighing from 18 to 24 gm. The animals were observed for 1 week after injection, and the LD_{50} values were calculated by the Litchfield-Wilcoxon method.[6]

Blood pressures of cats and dogs anesthetized with sodium secobarbital were recorded from the carotid artery with a mercury or membrane manometer.

Blood pressures of the trained unanesthetized dog were obtained by direct puncture of the femoral artery, using a Statham pressure transducer with a Grass polygraph as the recording unit. In the rat, blood pressure was measured without anesthesia by means of a photoelectric tensometer.

Results

Acute toxicity. Acute oral and intravenous LD_{50} values obtained in fasted female albino mice are shown in TABLE 1. The similarity of the oral and intravenous LD_{50} values for β-phenylethylhydrazine (No. 3), N-(β-phenylethyl)-N′-acetylhydrazine (No. 4), 1-acetyl-2-(α-ethylbenzyl) hydrazine (No. 12) and iproniazid demonstrated the excellent oral absorption of these substances. Some decrease in the completeness of oral absorption was noted in the remaining compounds.

Intravenous and oral administration of the MAO inhibitors to mice and rats caused stimulation of the central nervous system (hyperactivity, Straub tail response, piloerection, salivation, lacrimation, and convulsions). Most deaths occurred within 24 hours after drug administration. A species difference was noted, since single doses of β-phenylethylhydrazine (No. 3) failed to produce CNS stimulation in rabbits, cats, dogs, and monkeys.

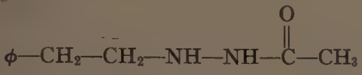
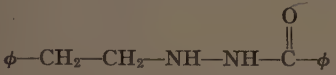
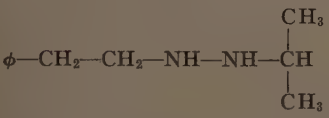
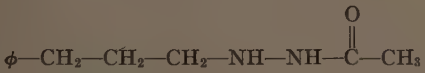
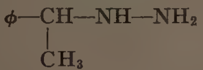
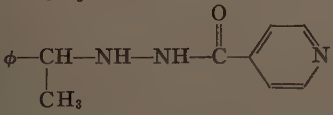
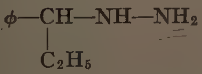
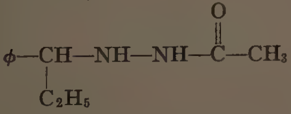
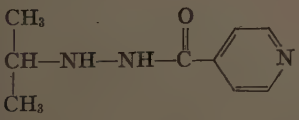
The data show that acetyl or benzoyl substitution on N′ of β-phenylethylhydrazine, as well as acetyl substitution on N′ of γ-phenylpropylhydrazine (No. 7), caused a reduction of toxicity.

Assessment of relative potency. The accumulation of mouse-brain serotonin served as an index of biological activity. Doses of the hydrazines required to produce a 50 per cent elevation of mouse-brain serotonin were expressed as ED_{50} values. Each ED_{50} value was calculated graphically from a dose-response curve. Peak responses of the rapid-acting compounds were measured at 3 hours after drug administration, and similar values for the substances showing a slower onset of action were determined at 6 hours (see TABLE 1).

Several relationships are evident from the data presented in TABLE 1. The

TABLE 1

LD$_{50}$ AND ED$_{50}$ VALUES OF HYDRAZINE DERIVATIVES IN MICE

Compound No.	Structure	LD$_{50}$ mg./kg.		ED$_{50}$* mg./kg.
		P.O.	I.V.	
1	$\phi{-}CH_2{-}CH{=}N{-}NH_2$	310 (292 to 329)†	Insoluble	>60
2	$\phi{-}CH_2{-}NH{-}NH_2$	69 (55 to 86)	72 (62 to 86)†	3
3	$\phi{-}CH_2{-}CH_2{-}NH{-}NH_2$	90 (86 to 96)	91 (85 to 97)	11
4	 $\phi{-}CH_2{-}CH_2{-}NH{-}NH{-}\overset{\overset{O}{\Vert}}{C}{-}CH_3$	180 (150 to 216)	130 (104 to 162)	28
5	 $\phi{-}CH_2{-}CH_2{-}NH{-}NH{-}\overset{\overset{O}{\Vert}}{C}{-}\phi$	390 (333 to 456)	Insoluble	50
6	 $\phi{-}CH_2{-}CH_2{-}NH{-}NH{-}\underset{\underset{CH_3}{\vert}}{\overset{\overset{CH_3}{\vert}}{CH}}$	212 (193 to 234)	55 (39 to 77)	15
7	$\phi{-}CH_2{-}CH_2{-}CH_2{-}NH{-}NH_2$	166 (139 to 206)	73 (65 to 91)	26
8	 $\phi{-}CH_2{-}CH_2{-}CH_2{-}NH{-}NH{-}\overset{\overset{O}{\Vert}}{C}{-}CH_3$	345 (324 to 371)	150 (130 to 174)	>50
9	 $\phi{-}\underset{\underset{CH_3}{\vert}}{CH}{-}NH{-}NH_2$	265 (213 to 328)	154 (141 to 168)	1.5
10		270 (221 to 329)	150 (120 to 188)	24
11	 $\phi{-}\underset{\underset{C_2H_5}{\vert}}{CH}{-}NH{-}NH_2$	231 (182 to 294)	103 (84 to 133)	4.5
12	 $\phi{-}\underset{\underset{C_2H_5}{\vert}}{CH}{-}NH{-}NH{-}\overset{\overset{O}{\Vert}}{C}{-}CH_3$	200 (174 to 234)	175 (158 to 194)	28
Iproniazid		950‡ (922 to 978)	725‡ (689 to 761)	90

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* Intraperitoneal dose necessary to produce 50 per cent elevation of brain serotonin 3 hours after drug administration. Values for compounds No. 2, No. 10, and iproniazid were determined at 6 hours.

† P = 0.95.

‡ Obtained from literature.[7]

potency of a monoamine oxidase inhibition does not necessarily parallel its acute toxicity. α-Phenylethylhydrazine (No. 9) is the most potent agent in this study, but is among the least toxic. Acyl substitution on N′ has decreased potency, as is evident from comparison of the following compounds: No. 3 with Nos. 4 and 5, No. 7 with No. 8, No. 9 with No. 10, and No. 11 with No. 12. Increasing the length of the aliphatic chain, that is, the distance between the phenyl and hydrazine portions of the molecule, has reduced activity. γ-Phenylpropylhydrazine (No. 7) is less active than β-phenylethylpropylhydrazine (No. 3), while benzylhydrazine (No. 2) is a more potent inhibitor than either of these compounds. Alpha-methyl substitution on benzylhydrazine increased activity as shown by the lower ED_{50} value for α-phenylethylhydrazine (No. 9). It should be noted that α-ethyl substitution (No. 11) was not advantageous.

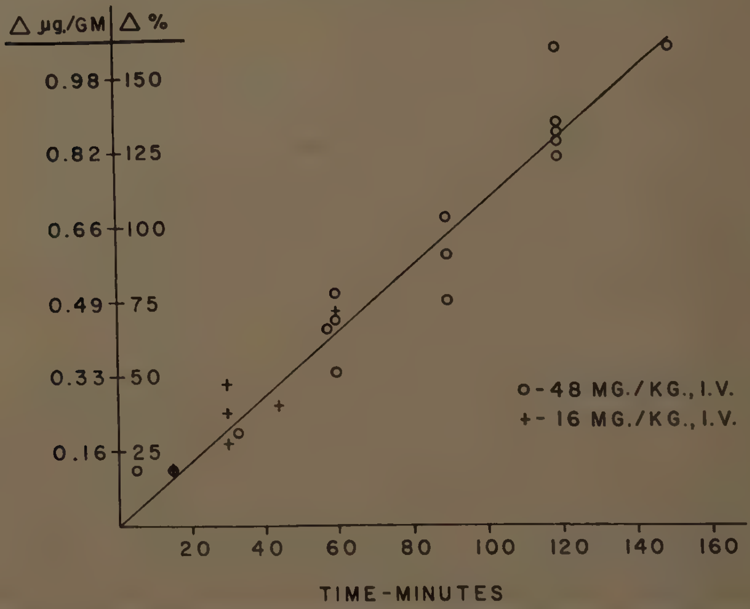

FIGURE 1. Rate of accumulation of mouse-brain serotonin following β-phenylethylhydrazine. Note that increasing the dose from 16 to 48 mg./kg. did not change the rate of serotonin accumulation. Increases over the basal control level (0.66 μg./gm. brain tissue) are demonstrated.

Effect on mouse-brain serotonin and MAO inhibition. It was of interest to subject β-phenylethylhydrazine to more intensive study, since the β-phenylethylamine structure may be considered as a parent compound of a large number of biologically active substances. FIGURE 1 illustrates the rapidity with which serotonin begins to accumulate in mouse brain after the injection of this inhibitor. The rate of serotonin accumulation was not increased when the intravenous dose of inhibitor was raised from 16 to 48 mg./kg. Doses approaching the lethal level of the compound did not change the rate.

Although there was no dose-response relationship regarding rate of serotonin accumulation, the size of the dose determined the ultimate levels achieved

(FIGURE 2). This response is typical of the rapid-acting, long-lasting MAO inhibitors.[8]

Levels of serotonin may well be related to MAO inhibition. FIGURE 3 demonstrates the return to pretreatment levels of mouse-brain serotonin and the recovery of MAO activity following a single dose (32 mg./kg., intraperitoneally) of β-phenylethylhydrazine. The long duration of action of this compound is clearly shown. At a time when MAO activity is more than 50 per cent inhibited, serotonin levels are near normal. It is thus conceivable that between 50 and 100 per cent inhibition of MAO may be required in order to elevate brain serotonin significantly.

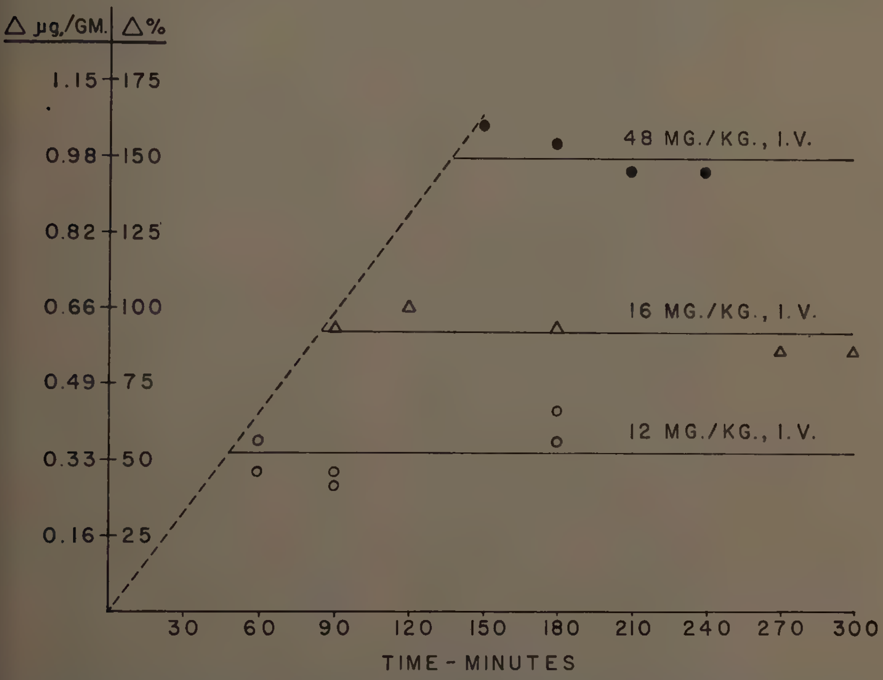

FIGURE 2. Effect of increasing doses of β-phenylethylhydrazine on the levels of mouse-brain serotonin. Note that a definite dose-response relationship exists in this respect. Serotonin control level is 0.66 µg./gm. brain tissue.

Action on blood pressure. The intravenous administration of β-phenylethylhydrazine induced a transient pressor response without the development of tachyphylaxis in the anesthetized cat and dog. On the contrary, repeated injections of the same dose produced a slight progressive increase in the response (FIGURE 4). The blood pressure of anesthetized dogs was not significantly altered by the oral administration of 10 to 25 mg./kg. of the compound. Daily administration of 5 mg./kg. of the hydrazine in capsule form had no effect on the blood pressure of trained normotensive dogs during a 2-week test period. A moderate antihypertensive effect was noted in *meta*-DCA hypertensive rats that received 2, 5, and 10 mg./kg. by the subcutaneous route daily for 5 days.

The most potent MAO inhibitor used in this study, α-phenylethylhydrazine, had no significant effect on mean arterial pressure of the anesthetized cat and dog when administered intravenously in doses up to 10 mg./kg. An increase in the chain length between the phenyl and hydrazine portions of the molecule from 2 to 3 carbons or a reduction to 1 carbon decreased pressor activity. Acyl substitution on N′ completely abolished the blood pressure effects, while an isopropyl substitution on N′ (No. 6) imparted vasodepressor properties. In general, it appears that structure-activity relationships for aromatic sympathomimetic amines[9] and this group of hydrazines are similar.

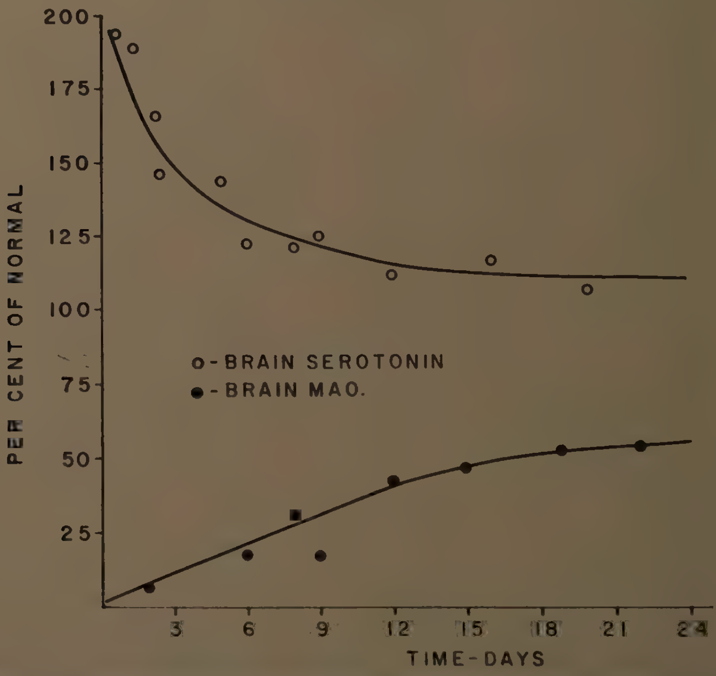

FIGURE 3. Relationship between mouse-brain serotonin levels and recovery of MAO activity following a single dose of β-phenylethylhydrazine (32 mg./kg., intraperitoneally).

Prolonged effect of β-phenylethylhydrazine. Earlier work demonstrated that the effects of MAO inhibition in mice persist for 2 to 3 weeks. It has also been demonstrated that reserpine administration to anesthetized cats and dogs pretreated with iproniazid results in a prompt and prolonged elevation of blood pressure and contraction of the nictitating membrane.[2, 3] Similar results have been obtained with β-phenylethylhydrazine in the same species.

FIGURE 5 illustrates the effect of reserpine on the blood pressure and nictitating membrane of an anesthetized cat 1 week after a single dose of β-phenylethylhydrazine (15 mg./kg., intraperitoneally). A concomitant widening of the palpebral fissure and mydriasis were observed. These responses, which are not evident in untreated control animals, are antagonized by adrenergic blocking drugs.

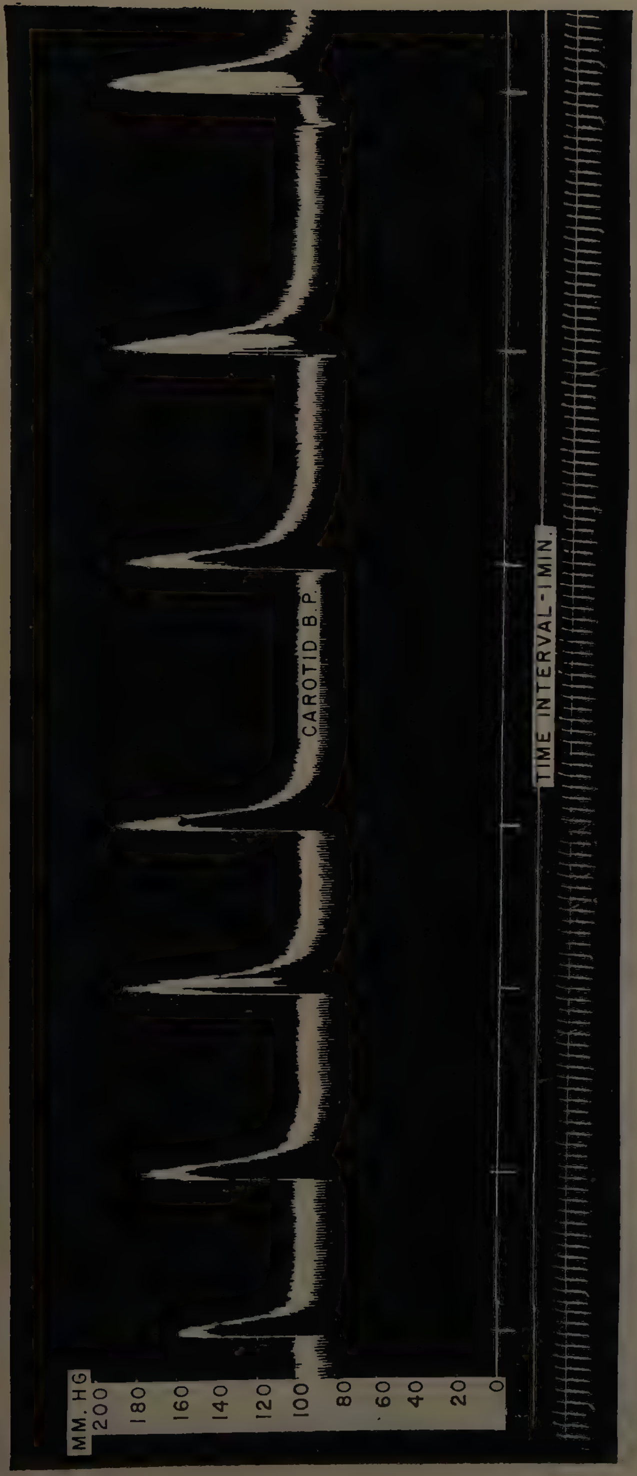

FIGURE 4. Effect of repeated doses of β-phenylethylhydrazine on blood pressure of the anesthetized dog. The upper signal marks intravenous injections of 0.6 mg./kg.

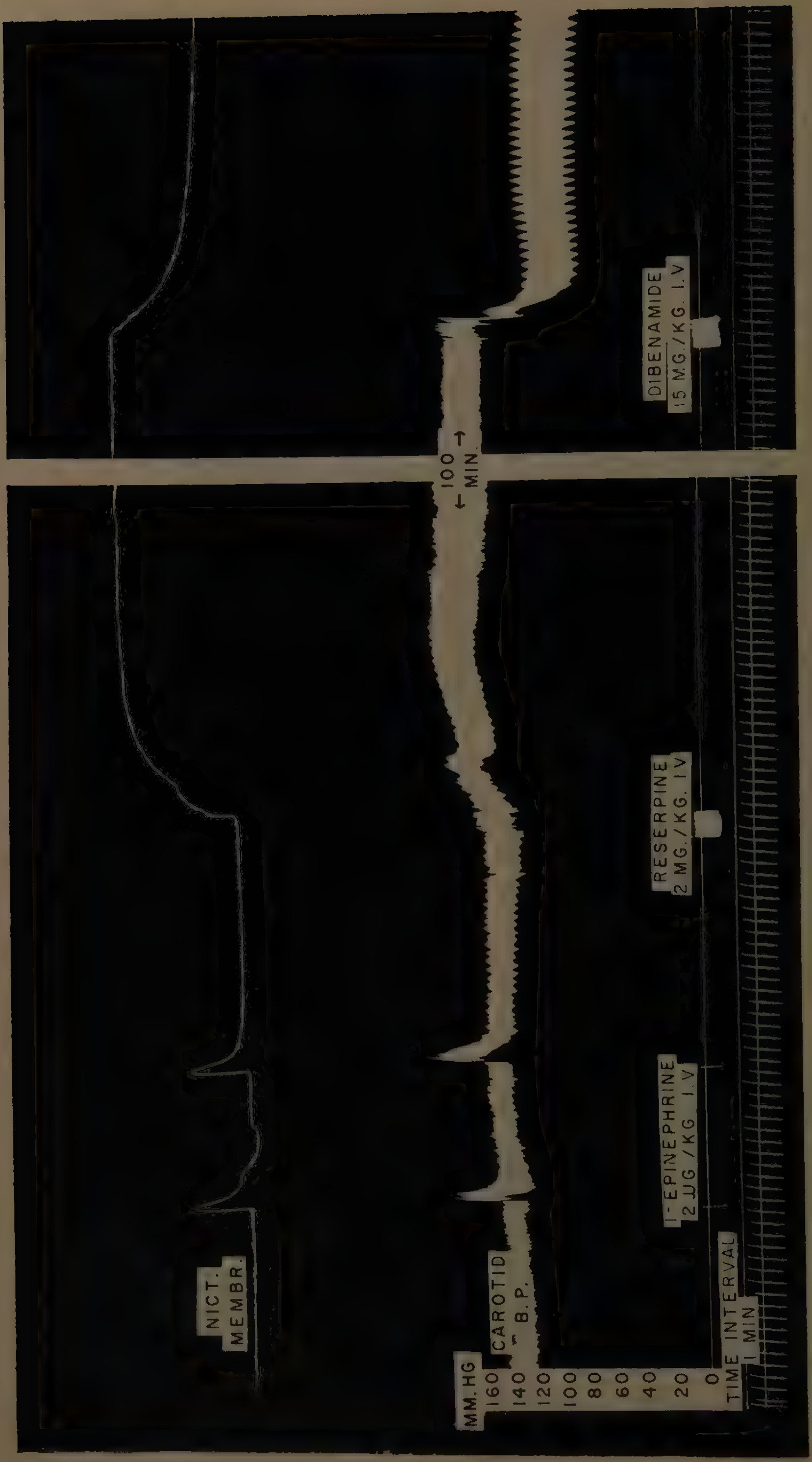

FIGURE 5. Effect of reserpine on blood pressure and nictitating membrane of the anesthetized cat one week after β-phenylethylhydrazine (15 mg./kg., intraperitoneally).

Action of β-phenylethylhydrazine in the pithed decerebrated cat. The pharmacological responses induced by β-phenylethylhydrazine in this preparation were similar to those observed in anesthetized cats. FIGURE 6 shows the usual transient responses elicited by the intravenous administration of epinephrine and the MAO inhibitor, as well as the effect of reserpine injected 1½ hours after the hydrazine derivative.

Alteration of the reserpine response by 1-desoxyephedrine. Reversible, short-acting MAO inhibitors such as 1-desoxyephedrine are capable of mimicking the activity of the hydrazine inhibitors in acute experiments. The reversal of the blood pressure response to reserpine in the anesthetized dog due to pretreatment with this drug is shown in FIGURE 7. It will be noted that a second injection of reserpine failed to cause a pressor response, as is the case with the hydrazine derivatives. Studies are in progress to investigate the "triggering mechanism" of various substances after MAO inhibition. The MAO-inhibiting activity of 1-desoxyephedrine is relatively short (2 to 8 hours) in comparison to the potent hydrazine derivatives.

Effect of β-phenylethylhydrazine on the reserpine response in adrenalectomized dogs. β-Phenylethylhydrazine produced its usual transient pressor response in anesthetized dogs that had been adrenalectomized 1 hour prior to the intravenous administration of the drug. The typical reversal of the blood pressure response to a subsequent injection of reserpine was evident.

Action on the isolated intestine. Concentrations of β-phenylethylhydrazine as high as 50 μg./ml. of tissue bath had no effect on motility of the isolated guinea pig ileum or on spasms induced by acetylcholine and histamine.

Summary

β-Phenylethylhydrazine and a group of related compounds were investigated for structure-activity relationships with respect to MAO inhibition and pharmacological properties.

The relative ability of this group of compounds to inhibit MAO did not always correlate with their LD_{50} values. The onset of action of the hydrazines was rapid and their duration prolonged (2 to 3 weeks). Results obtained with β-phenylethylhydrazine indicate that 50 to 100 per cent inhibition of MAO is necessary in order to achieve significant elevation of brain serotonin.

Intravenous administration of β-phenylethylhydrazine produced a transient pressor response in the anesthetized animal. Oral administration to either the anesthetized or unanesthetized normotensive dog did not produce this effect. Definite structure-activity relationships regarding pressor properties of β-phenylethylhydrazine and related compounds were established.

β-Phenylethylhydrazine reversed reserpine actions in the pithed decerebrated animal, as it did in the normal anesthetized animal. These effects could be induced either acutely or 1 week after administration of the MAO inhibitor. Comparative studies were conducted with the relatively weak, short-acting MAO inhibitor, 1-desoxyephedrine.

Acknowledgment

We express our sincere appreciation to Jonas A. Gylys for his valuable assistance in compiling and analyzing data.

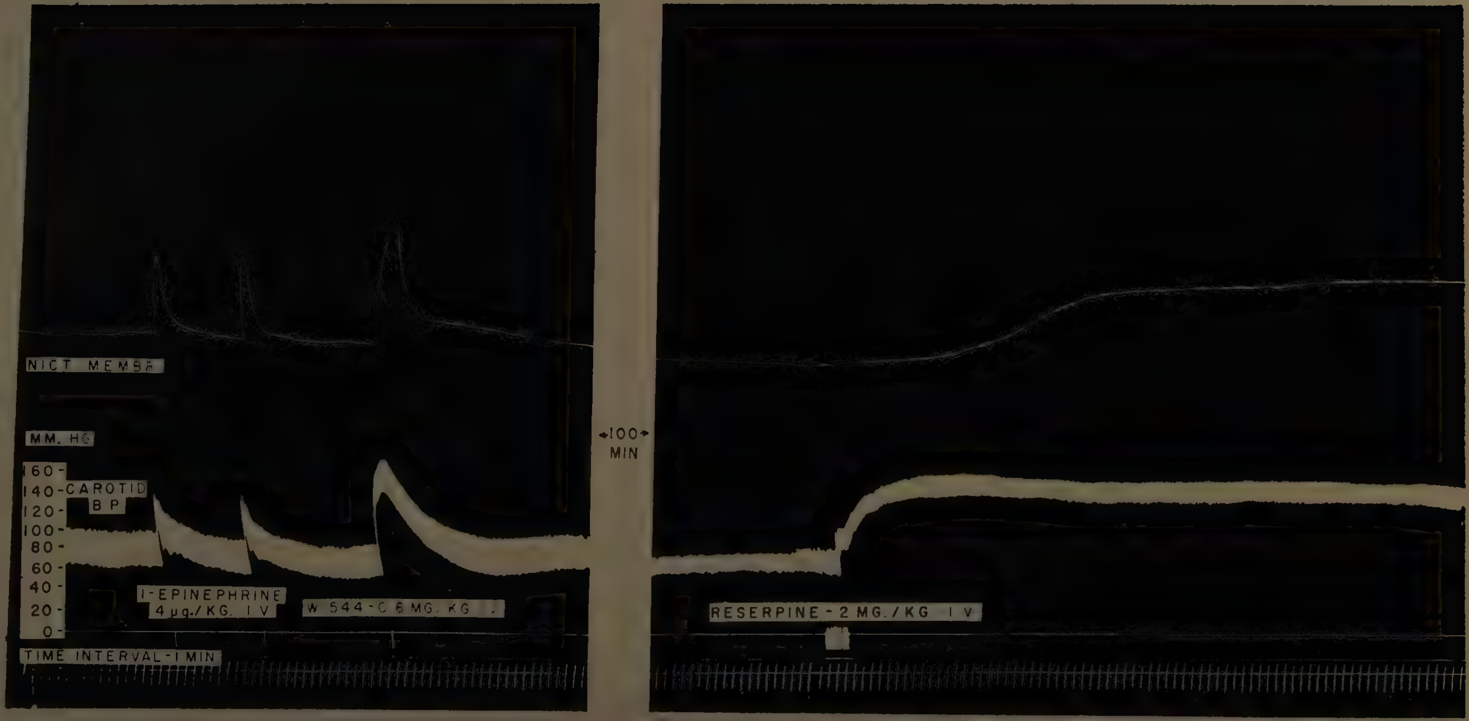

FIGURE 6. Effect of β-phenylethylhydrazine on blood pressure and nictitating membrane of the pithed decerebrated cat.

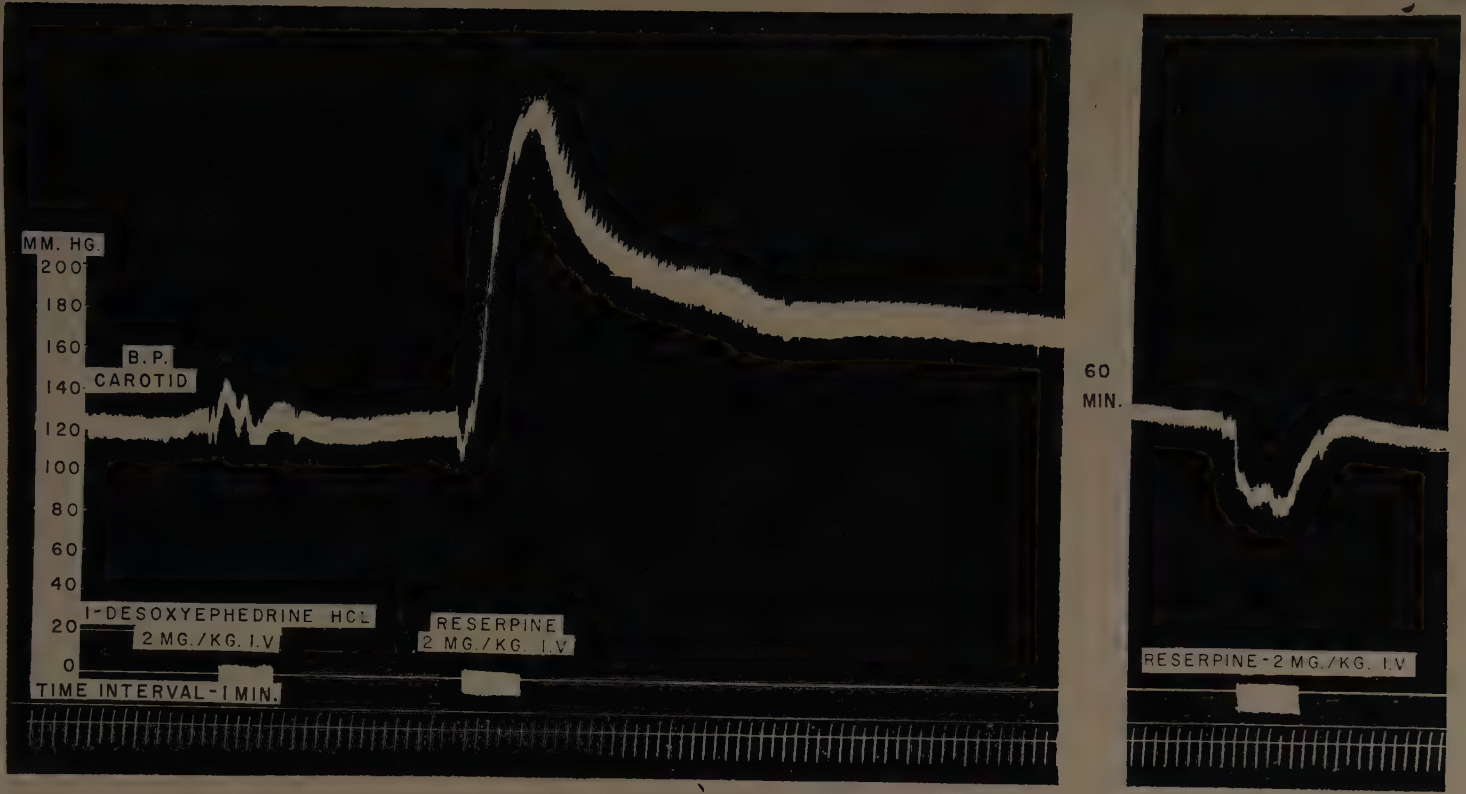

FIGURE 7. Effect of 1-desoxyephedrine on reserpine-induced blood pressure responses in the anesthetized dog.

References

1. Brodie, B. B., A. Pletscher & P. A. Shore. 1956. Possible role of serotonin in brain function and in reserpine action. J. Pharmacol. Exptl. Therap. **116:** 9.
2. Chessin, M., B. Dubnick, E. R. Kramer & C. C. Scott. 1956. Modifications of pharmacology of reserpine and serotonin by iproniazid. Federation Proc. **15:** 409.
3. Chessin, M., E. R. Kramer & C. C. Scott. 1957. Modifications of the pharmacology of reserpine and serotonin by iproniazid. J. Pharmacol. Exptl. Therap. **119:** 453.
4. Udenfriend, S., H. Weissbach & C. T. Clark. 1955. The estimation of 5-hydroxytryptamine (serotonin) in biological tissues. J. Biol. Chem. **215:** 337.
5. Bogdanski, D. F., A. Pletscher, S. Udenfriend & B. B. Brodie. 1956. Identification and assay of serotonin in brain. J. Pharmacol. Exptl. Therap. **117:** 82.
6. Litchfield, Jr. J. T. & F. Wilcoxon. 1949. A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exptl. Therap. **95:** 99.
7. Benson, W. M., P. L. Stefko & M. D. Roe. 1952. Pharmacologic and toxicologic observations on hydrazine derivatives of isonicotinic acid (Rimifon, Marsilid). Am. Rev. Tuberc. **65:** 376.
8. Dubnick, B., G. Leeson, M. Chessin & C. C. Scott. 1958. Some factors influencing the rate of accumulation of serotonin in mouse brain after inhibition of monoamine oxidase. Abstracts. Fall Meeting. Am. Soc. Pharmacol. Exptl. Therap. : 10.
9. Goodman, L. S. & A. Gilman. 1955. Drugs stimulating structures innervated by adrenergic nerves. Chapt. 23. *In* The Pharmacological Basis of Therapeutics. : 479–480. Macmillan. New York, N. Y.

INTERACTION OF MONOAMINE OXIDASE INHIBITORS WITH PHYSIOLOGICAL AND BIOCHEMICAL MECHANISMS IN BRAIN

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Accumulating evidence indicates that monoamine oxidase (MAO) is important in the metabolism of serotonin (5-HT) and norepinephrine (NE). Since these amines are substances of high biological activity and have been implicated in brain function, it becomes of great interest to see whether the antidepressant effects elicited by MAO inhibitors are related to changes in the brain levels of these amines.[1-3]

The effect of a single dose of iproniazid (Marsilid)* (100 mg./kg.) on the brain amines is shown in FIGURE 1.[4, 5] The level of 5-HT rises over a period of 6 hours until it reaches a plateau about twice normal. The level of NE rises more slowly and to a somewhat lesser degree. No obvious pharmacological signs result from a single dose of the drug.

Daily doses of 25 mg. of iproniazid/kg. also elevate 5-HT levels, which reach a plateau 2 to 3 times normal in 2 days, while NE again rises more slowly but finally, by the fourth day reaches a maximum level (TABLE 1), which is somewhat higher than that obtained from the single large dose. At this time, the rabbits show definite sympathomimetic signs, including mydriasis, constriction of the blood vessels in the ear, and an increased motor activity. These effects disappear when the NE levels decline on cessation of drug administration, even though the 5-HT levels are still elevated.

FIGURE 2 shows the effect of a single dose (2 mg./kg.) of the potent MAO inhibitor JB-516 (phenylisopropylhydrazine).[6, 7] The 5-HT rises more rapidly than after iproniazid, reaching its maximum level in about 1 hour; the NE levels again rise more slowly and to a lesser degree. Here, too, no pharmacological effects are evident. The administration of JB-516 in daily doses of 2 mg./kg. elevates the 5-HT levels no higher than does the single dose but, by the fourth day, it elicits appreciably higher NE levels (TABLE 2). The animals now exhibit the same sympathomimetic signs as seen after repeated doses of iproniazid. When the drug administration is terminated the excitation again disappears when the NE levels decline, although the 5-HT levels are still elevated.

The same pattern of response is seen with JB-835 (phenylisobutylhydrazine), another potent MAO inhibitor that has activity comparable to that of JB-516 (FIGURE 3, TABLE 3).

These results suggest that the rise in 5-HT is not the important factor in the excitation caused by MAO inhibitors, but that NE might be. Further evidence of this is found in the failure of MAO inhibitors to produce psychomotor activity in cats even after daily administration of the drugs for a period of 7 days. In this species the inhibitors induce a pronounced increase in brain

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* Hoffmann-La Roche.

5-HT, but have little or no effect on NE levels (TABLE 4). It is not known why the brain levels of NE are not affected in cats. The turnover of NE may be so slow that the amine diffuses from the brain as rapidly as it is formed. Alternatively, an enzyme other than MAO may catalyze the metabolism of the amine. Similar experiments in the dog also show a marked rise in brain 5-HT but not NE, even after daily administration of JB-516 for 12 days (TABLE 5).

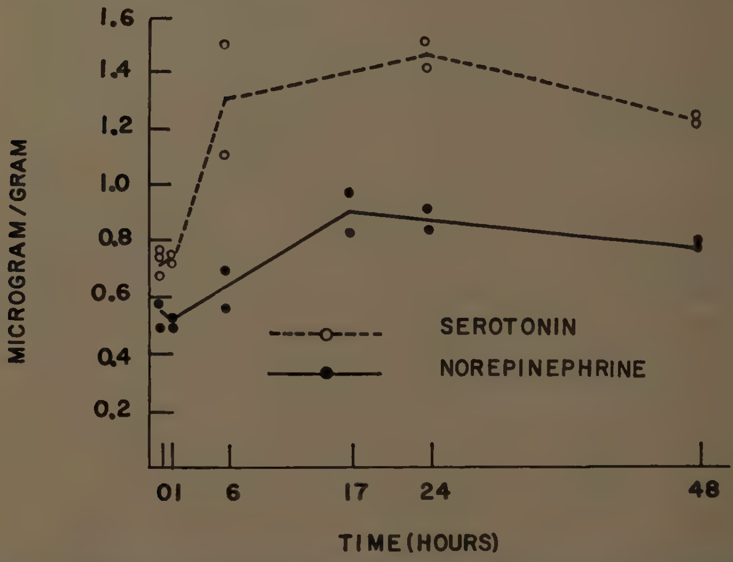

FIGURE 1. Effect of a single large dose of iproniazid (100 mg./kg.) on 5-HT and NE levels in rabbit brain stem. 5-HT and NE were analyzed according to methods previously described.[4, 5]

TABLE 1
EFFECT OF DAILY SUBCUTANEOUS INJECTION OF IPRONIAZID (25 MG./KG.) ON 5-HT AND NE CONTENT OF RABBIT BRAIN STEM

Time (Days)	5-HT µg./gm.	NE µg./gm.
0	0.65	0.55
1	1.0	0.55
2	1.35	0.92
4	1.40	1.00
6	1.55	1.00

Studies with JB-807 (1-phenylisopropyl-2-isopropylhydrazine), however, cast some doubt on the possibility that the excitation evoked by MAO inhibitors is mediated through an increase in brain NE. This MAO inhibitor is about as active *in vitro* as iproniazid.

After a single dose (10 mg./kg.) of JB-807 to rabbits, there is a delay of about 2 hours before an elevation in brain serotonin is seen. NE levels rise to an even lesser extent (FIGURE 4, TABLE 6). The high activity of this compound *in vivo*, as well as its delayed activity, suggests that it may act through a metabolic product.

Thus, it is not possible definitely to ascribe the central actions of MAO inhibitors to the increase in brain NE or 5-HT. However, it must also be borne in mind that the apparent excitation observed in the animals is not necessarily a reflection of the antidepressant "pyschic energizing" effect of the drugs in man.

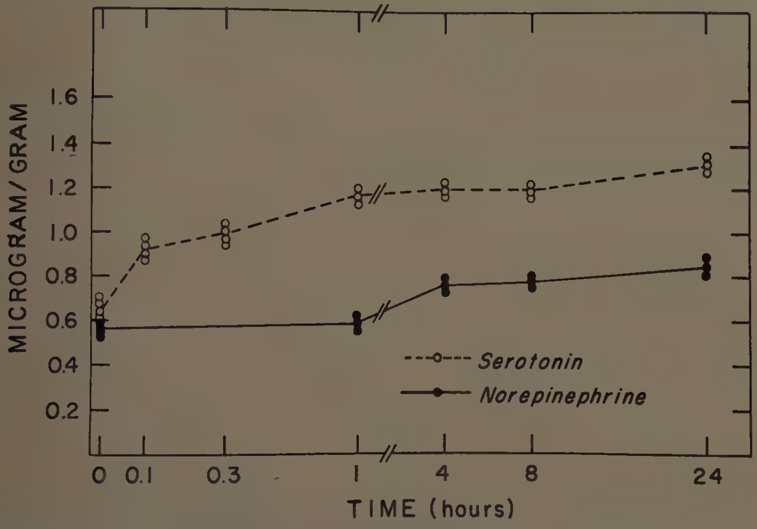

FIGURE 2. Effect of a single dose of JB-516 (2 mg./kg.) on 5-HT and NE levels in rabbit brain stem.

TABLE 2

BRAIN STEM AMINE LEVELS AND PHARMACOLOGICAL EFFECTS INDUCED BY JB-516 (2 MG./KG.): COMPOUNDS ADMINISTERED DAILY FOR 5 DAYS AND THEN DISCONTINUED

Day	Behavior	Pupil	Ear vessel	5-HT µg./gm.	NE µg./gm.
0	Normal	Normal	Normal	0.72	0.59
1	Normal	Normal	Normal	1.16	0.86
3	Normal	Normal	Normal	1.17	0.82
4	Hyperactive	Mydriasis	Constricted	1.40	1.02
5	Hyperactive	Mydriasis	Constricted	1.46	1.11
9	Normal	Normal	Normal	1.49	0.76

The effect of MAO inhibitors on amine levels in other tissues. In general, MAO inhibitors do not elevate the levels of 5-HT or NE in peripheral tissues. An exception is platelets, whose 5-HT levels rise after the administration of MAO inhibitors.[8, 9] Since 5-HT is not formed in platelets, the 5-HT must have been picked up by these cells from other tissues. NE levels in guinea pig heart have also been reported to increase following repeated doses of iproniazid.[10]

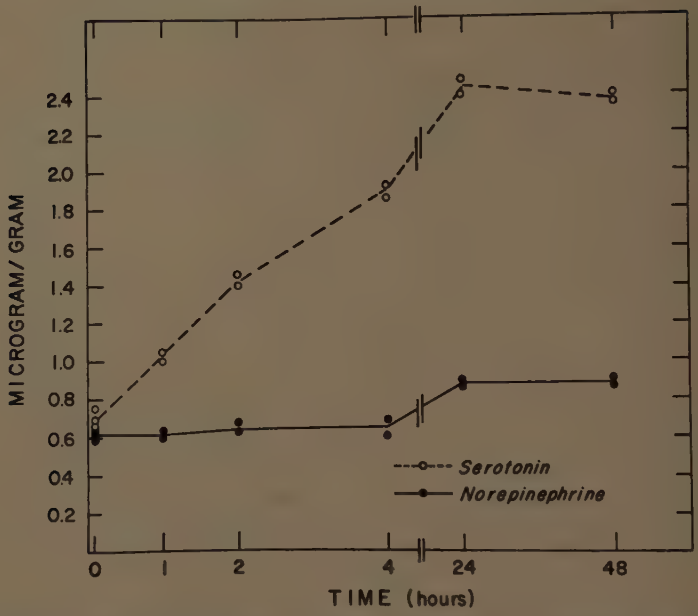

FIGURE 3. Effect of a single dose of JB-835 (10 mg./kg.) on 5-HT and NE levels in rabbit brain stem.

TABLE 3
BRAIN STEM AMINE LEVELS AND PHARMACOLOGICAL EFFECTS INDUCED BY DAILY ADMINISTRATION OF JB-835 (10 MG./KG.)

Day	Behavior	Pupil	Ear vessel	5-HT µg./gm.	NE µg./gm.
0	Normal	Normal	Normal	0.74	0.62
1	Normal	Normal	Normal	2.25	0.73
2	Normal	Normal	Normal	1.90	0.88
3	Normal	Normal	Normal	2.46	1.13
4	Normal	Normal	Normal	2.22	1.15
5	Hyperactive	Mydriasis	Constricted	2.43	1.21

TABLE 4
EFFECT OF DAILY DOSES OF JB-516 (2 MG./KG. SUBCUTANEOUSLY) ON BRAIN STEM LEVELS OF 5-HT AND NE IN THE CAT

Time	Concentration µg./gm.	
	5-HT	NE
0	0.79	0.62
4 hours	1.46	0.77
1 day	1.92	0.85
3 days	1.47	0.67
4 days	1.94	0.54
7 days	2.90	0.80

Turnover of brain amines. The rapid action of JB-516 has been utilized in an attempt to measure the rates of synthesis of 5-HT and NE in rabbit brain. The rate is extremely rapid for 5-HT, 3 mg./kg. of JB-516 inducing a 50 per cent rise 10 to 15 min. after intravenous injection.[11] The rise is considerably slower for NE, a 50 per cent rise requiring several hours. Since MAO appears to be the enzyme largely responsible in the rabbit for the inactivation of brain NE,[12] it would appear that NE in brain is synthesized much more slowly than is 5-HT. Preliminary results *in vitro*[11] had indicated that JB-516 might block

TABLE 5
EFFECT OF DAILY DOSES OF JB-516 (2 MG./KG.) ON LEVELS OF 5-HT AND NE IN BRAIN STEM OF THE DOG

Day	Concentration µg./gm.	
	5-HT	NE
0	0.75	0.54
5	3.83	0.58
8	3.76	0.60
9	4.10	0.34
12	5.05	0.47

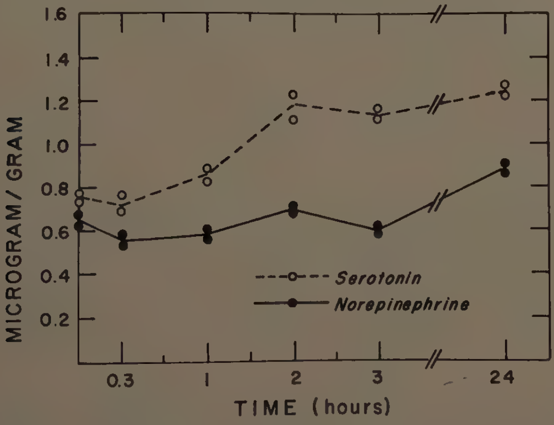

FIGURE 4. Effect of a single dose of JB-807 (10 mg./kg.) on 5-HT and NE levels in rabbit brain stem.

TABLE 6
BRAIN STEM AMINE LEVELS AND PHARMACOLOGICAL EFFECTS INDUCED BY DAILY ADMINISTRATION OF JB-807 (4 MG./KG.) TO RABBITS

Day	Behavior	Pupil	Ear vessel	5-HT µg./gm.	NE µg./gm.
0	Normal	Normal	Normal	0.62	0.51
9	Normal	Normal	Normal	1.55	0.90
18	Normal	Normal	Normal	2.49	1.03
30	Normal	Normal	Normal	2.68	1.15

NE synthesis by inhibiting the decarboxylation of dihydroxyphenylalanine (DOPA), but subsequent studies *in vivo* have shown that the drug does not interfere with this reaction in rabbit brain.

It is noteworthy that while JB-516 causes an almost immediate rise in brain serotonin levels, iproniazid causes a relatively slow rise. However, when the brains of animals given either JB-516 or iproniazid are removed at various times after drug administration and homogenates tested for their MAO activity, a rapid abolition of activity is observed. These findings suggest that both drugs readily penetrate the brain, but that JB-516 reaches the actual site of MAO activity at a faster rate than iproniazid.

Acknowledgment

The iproniazid (Marsilid) used in these investigations was supplied by Hoffmann-La Roche Inc., Nutley, N. J.

References

1. Spector, S., D. Prockop, P. A. Shore & B. B. Brodie. 1957. Science. **127:** 704.
2. Pletscher, A. 1957. Schweiz. Med. Wochschr. **87:** 1532.
3. Udenfriend, S., H. Weissbach & D. F. Bogdanski. 1957. Ann. N. Y. Acad. Sci. **66**(3)**:** 602.
4. Bogdanski, D. F., A. Pletscher, B. Brodie & S. Udenfriend. 1956. J. Pharmacol. **117:** 82.
5. Shore, P. A. & J. S. Olin. 1958. J. Pharmacol. **122:** 295.
6. Biel, J., A. Drukker, P. A. Shore, S. Spector & B. Brodie. 1958. J. Am. Chem. Soc. **80:** 1519.
7. Horita, A. 1958. J. Pharmacol. **122:** 176.
8. Shore, P. A., L. Gillespie, S. Spector & B. B. Prockop. 1958. Naturwiss. **45:** 340.
9. Pletscher, A. & A. Bernstein. 1958. Nature. **181:** 1133.
10. Pletscher, A. 1958. Experientia. **16:** 73.
11. Brodie, B. B., S. Spector, R. Kuntzman & P. A. Shore. 1958. Naturwiss. **45:** 243.
12. Shore, P. A., J. A. R. Mead, R. Kuntzman, S. Spector & B. B. Brodie. 1957. Science. **126:** 1063.

Discussion of the Paper

H. E. Himwich, E. Costa, G. R. Pscheidt, and W. G. Van Meter (*Galesburg State Research Hospital, Galesburg, Ill.*): The excellent paper of Brodie and his colleagues is concerned with the interaction of monoamine oxidase inhibitors with brain mechanisms. In this brief discussion we shall examine the effect of iproniazid (Marsilid) on one brain mechanism, namely, the ability of this drug to alter the function of the midbrain reticular formation. This structure regulates EEG alerting and is a factor in the production of behavioral arousal. The EEG end points for either the activated or alert brain and the drowsy or sleepy brain of the rabbit are distinct. Figure 1 shows the EEG of a rabbit that is in the alert state at the beginning of the record, as evidenced by low-amplitude fast waves seen especially in the left motor and right limbic leads, as well as regular 4 to 6 cps in the hippocampus. This alert EEG pattern is followed by one of lesser alertness or of drowsiness, with a rush of rapid 14-cps brain waves. Then there is a second, shorter period of alerting, followed by a longer one of drowsiness. These are spontaneous changes in the EEG, but peripheral stimulation evoked by touching the rabbit or pinching its toe can also cause alerting. Our purpose is to determine the EEG effects of irponiazid and to compare these effects with the concentrations of the catecholamines and serotonin, measured by the methods of Shore and Olin[1] and of Garven,[2] respectively. In this report we shall not present the EEG changes due to iproniazid, but the arousal and blocking of the activated systems caused by the drug are similar to those observed in the spontaneous variations of the EEG.

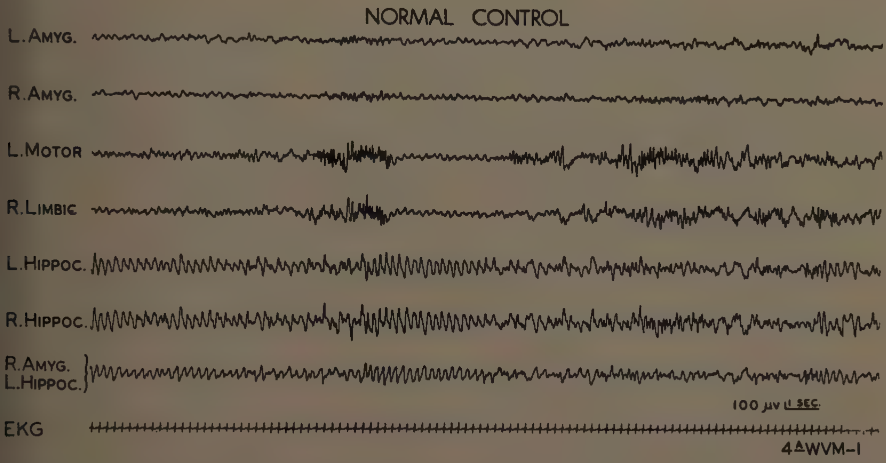

FIGURE 1. Control electroencephalogram of normal rabbit. The figure begins with a period of EEG activation most noticeable in the left motor and right limbic leads, but also in the hippocampus, followed by a shorter interval of lessened alertness. A second period of arousal occurs next and is succeeded by a much longer one with the drowsy pattern. Monopolar leads are left amygdaloid, right amygdaloid, left motor, right limbic, left hippocampus, and right hippocampus. Bipolar leads are right amygdaloid and left hippocampus. The bottom tracing contains the electrocardiograph record.

TABLE 1

CATECHOLAMINES: $\mu\mu$G./GM. RABBIT BRAIN

	Hippocampus	Midbrain	Pons-medulla
Control	200	320	260
Iproniazid 500 to 900 mg./kg.: activation	170	190	210
Iproniazid 25 mg./kg./day: no activation	370	710	560

Catecholamines, $\mu\mu$g./gm. in rabbit brain parts. A single large dose of iproniazid, 500 to 900 mg./kg., does not raise the catecholamine content within 1 hour after injection of the drug. On the other hand, a smaller dose of iproniazid (25 mg./kg.) given over a period of 5 days effectively increases catecholamines in the 3 brain parts studied: hippocampus, midbrain, and pons-medulla.

TABLE 1 shows the action of iproniazid on the catecholamine concentration in rabbit brain parts. A single dose of iproniazid, between 500 and 900 mg./kg., produced EEG activation in approximately one-half hour. There was no significant change, however, in catecholamines, even though the alerting was continued for another one-half hour before the brain was extirpated. On the other hand, the daily administration of iproniazid (25 mg./kg.) over a period of 5 days evoked a large increase in catecholamine content. Nevertheless, there was no enduring change in the EEG, whether to an alert or sleeping pattern. As shown in TABLE 2, however, the rise in catecholamines was accompanied by a similar one in serotonin. The lack of change in the electric pattern may be a result of 2 simultaneous neurohormonal alterations.

The results of a single dose of iproniazid (500 mg./kg.) on the serotonin concentrations in rabbit brain parts are seen in TABLE 2. Although the increase in serotonin is slight, EEG activation occurred. It is probable that this increase of serotonin is not related to the activation. A larger rise in the concentration of serotonin occurred without activation of the EEG after the intravenous injection of 5-hydroxytryptophan (75 mg./kg.).[3] When, however, iproniazid, 25 mg./kg., was administered daily for 5 days, an even greater augmentation of serotonin was observed and, without a prevailing EEG pattern for the brain waves, changed from sleep to alerting in accordance with appropriate environmental stimuli. Thus, the simultaneous increases of catecholamines and serotonin seem to be responsible for lifting the EEG pattern produced by a rise in serotonin only.

TABLE 2

SEROTONIN CONCENTRATIONS IN RABBIT BRAIN $\mu\mu$G./GM. TISSUE

	Telencephalon	Hippocampus	Midbrain	Pons-medulla
Saline	265	259	663	582
Iproniazid 500 mg./kg.: activation	332	259	897	—
5-HTP 75 mg./kg.: prevents activation	741	942	1836	1332
Iproniazid 25 mg./kg./day: no activation	987	1701	1554	—

Serotonin concentration in rabbit brain parts ($\mu\mu$g./gm.). A single dose of iproniazid (500 mg./kg.) increases serotonin concentrations of 2 brain parts only slightly. On the other hand, the injection of 5-hydroxytryptophan (75 mg./kg.) induces an increase in serotonin concentrations of the telencephalon, hippocampus, midbrain, and pons-medulla, associated with an inhibition of the usual peripheral stimulation-induced activation of the EEG. The administration of iproniazid (25 mg./kg.) once daily for 5 days raises the concentrations of brain serotonin.

In conclusion, we may say that a large single dose of iproniazid evoked EEG alerting without any associated changes in serotonin or catecholamines. A moderate rise in serotonin alone depresses the reticular formation and evokes an enduring sleep pattern. On the other hand, the simultaneous increases of serotonin and catecholamines in the brain do not evoke a characteristic EEG pattern, but leave the brain with an apparently normal sensitivity to environmental stimuli.

References

1. SHORE, P. A. & J. S. OLIN. 1958. Identification and chemical assay of norepinephrine in brain and other tissues. J. Pharmacol. Exptl. Therap. **122:** 295–300.
2. GARVEN, J. D. 1956. The estimation of 5-hydroxytryptamine in the presence of adrenaline. Brit. J. Pharmacol. **11:** 66–70.
3. COSTA, E. & F. RINALDI. 1958. Biochemical and electroencephalographic changes in the brain of rabbits injected with 5-hydroxytryptophan (influence of chlorpromazine premedication). Am. J. Physiol. **194:** 214–220.

EFFECTS OF AMINE OXIDASE INHIBITORS ON CEREBRAL CORTICAL RESPONSES TO EPINEPHRINE*

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Recent developments have shown that the adrenal hormone epinephrine has the property of acting directly on neuronal aggregates of the central nervous system.[1-3] Clinical observations have been reported that epinephrine can initiate emotional changes such as anxiety and mental confusion.[4-6] Combined physiological and clinical data thus support the working hypothesis that the reactivity of cerebral structures to epinephrine may constitute a major factor in normal cerebral functions and, possibly, in cerebral disorders.

An experimental approach based on a recently developed technique strengthens this hypothesis. This technique consists of an evaluation of systemic blood-pressure changes that follow the topical application of epinephrine to the frontoparietal area of the exposed cerebral cortex of the anesthetized rabbit.[7]

The experiments were performed with adult rabbits anesthetized with urethane. The cerebral hemispheres were exposed, the dura opened, and mean systemic arterial blood pressure was measured via a cannula in the left femoral artery. Next, a very small piece of filter paper (3 to 5 mm. square), moistened with a freshly prepared 5 per cent solution of epinephrine bitartrate in Ringer's solution, was placed on the frontoparietal zone of the cortex. After a very short contact (15 to 30 sec.) the systemic blood pressure rose 30 to 60 mm. Hg, maintained a maximum for 0.5 to 2 min., and then decreased to its initial value. Subsequent applications were made at 10-min. intervals.

The size and duration of the cortically induced pressor effects depend upon the area treated. They were maximal after local administration of epinephrine on the median frontoparietal cortex, smaller and more transient on the frontal area, and generally negligible on the temporal and occipital regions. This cortically induced pressor effect could also be elicited in rats, guinea pigs, and cats.

There was no effect on the systemic blood pressure upon the application to the cerebral cortex of Ringer's solution, 5-hydroxytryptamine, or histamine. Electric stimuli produced a small and transient hypotension. Applications of norepinephrine produced a pressor response that was much smaller than the pressor response due to epinephrine.

One of the characteristics of this phenomenon is the fact that the blood-pressure response increases gradually after repeated applications of epinephrine (at 10-min. intervals) on the same area. The hypertension engendered by cortical application of epinephrine is due to a mechanism that is essentially different from that produced by intravenous injections of the same hormone. Both effects could be differentiated through an intravenous administration of a massive dose of epinephrine (750 μg.), after which the peripheral effect of that substance was enhanced, whereas the cortical pressor response was noticeably reduced or abolished.

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† Senior Research Fellow of the United States Public Health Service.

Further detailed pharmacodynamic analysis and transection studies at various levels have shown that the cortically induced pressor reaction is caused by an unknown hypertensive agent released from the hypothalamus.[8] It can then be summarized that the humoral stimulus produced by epinephrine on the cerebral cortex was transmitted by neuronal elements to a hypothalamic center capable of secreting a substance with hypertensive activity. The hypothalamic area that constitutes a projection zone of the autonomic nervous system contains relatively high concentrations of neurohumors such as acetylcholine, 5-hydroxytryptamine, epinephrine, norepinephrine, and substance P, as well as enzyme systems concerned with their synthesis and breakdown. It thus seemed of interest to investigate possible modifications of the cortically applied epinephrine pressor response by systemic premedication with centrally acting drugs, some of which are supposed to act at hypothalamic levels. Some of the drugs studied are listed in TABLE 1.

TABLE 1

EFFECTS OF VARIOUS DRUGS ON THE CORTICAL EPINEPHRINE PRESSOR RESPONSE

Drug	Pharmacological action	Catecholamine content of brain	Cortically applied epinephrine pressor response
Piperoxan	Adrenergic blocking agent	—	No change
Dihydroergotamine	Adrenergic blocking agent	—	Abolished
Chlorpromazine	Tranquilizer, adrenergic blocking agent	No change	Abolished
Azacyclonol	Tranquilizer	No change	No change
Meprobamate	Tranquilizer	No change	No change
Reserpine	Tranquilizer	Decreased	Augmented
Tetrabenazine RO 1-9569	Tranquilizer	Decreased	Augmented
RO 4-1038	Amine oxidase inhibitor	Increased	Diminished
RO 4-1018	Amine oxidase inhibitor	Increased	Diminished
Iproniazid	Amine oxidase inhibitor	Increased	Diminished
Isoniazid	Tuberculostatic	No change	No change
Pipradrol	Stimulant	—	No change
Arecoline	Parasympathomimetic	—	Diminished
L-DOPA	—	Increased	Diminished

Rabbits systemically pretreated with reserpine (1 mg./kg.) consistently showed an augmented pressor response to cortically applied epinephrine (FIGURE 1).[9] This augmentation of the pressor response was of such magnitude that essentially normal pressor responses were obtained with 0.5 per cent and even 0.1 per cent solutions of epinephrine bitartrate.

We have previously reported that the pressor response to topical application of epinephrine to the cerebral cortex was abolished, diminished, or even slightly reversed in rabbits that had been pretreated with serum from some schizophrenic patients.[10, 11] These results were found in approximately 65 per cent of the schizophrenic patients studied. This modification of the pressor effect was never found in rabbits pretreated with serum from normal volunteers. Furthermore, rabbits receiving serum from schizophrenic patients showed an increased catecholamine content of the brain and especially of the hypothalamus.[12] Reserpine is capable of decreasing the catecholamine content of the brain[13] and augmenting the cortical epinephrine pressor response; on the other hand, serum from schizophrenic patients increased the catecholamine content of the brain and decreased or abolished the cortical epinephrine pressor response. Thus, as a working hypothesis, we considered the existence of a functional correlation between the cortical response to epinephrine and the catecholamine level of the hypothalamic area.

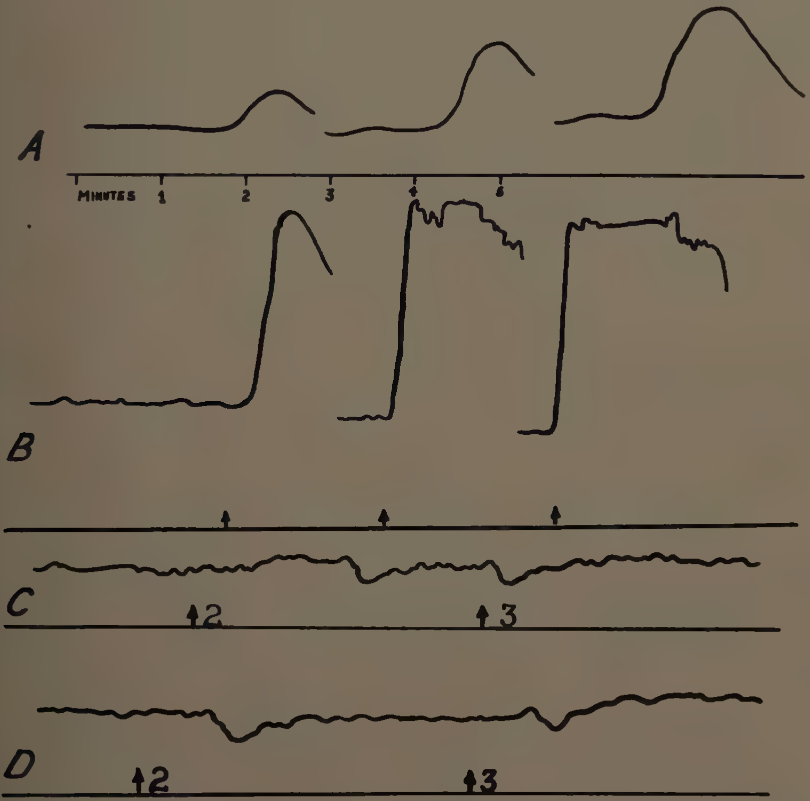

FIGURE 1. Rabbit blood pressure. (*A*) Blood pressure tracing showing 3 applications of epinephrine at 10-min. intervals on the frontoparietal area of the cerebral cortex of a rabbit; (*B*) blood pressure tracing illustrating the cardiovascular response to 3 applications of epinephrine to the same cortical area in a rabbit pretreated 4 hours previously with reserpine (1 mg./kg.); (*C*) blood pressure tracing illustrating the cardiovascular response to the second and third applications of epinephrine to the same cortical area in a rabbit pretreated with serum from a schizophrenic patient; (*D*) blood pressure tracing illustrating the cardiovascular response to the second and third application of epinephrine to the same cortical area in a rabbit pretreated 16 hours previously with iproniazid (100 mg./kg.).

Results of Experiments with Amine Oxidase Inhibitors

According to recent observations of Spector and his co-workers,[14] iproniazid (Marsilid) administered systemically to rabbits was capable of increasing the norepinephrine content of the brain stem in these animals. The mechanism of this increase is considered to be the result of the inhibition in the brain of monoamine oxidase, which is the enzyme concerned with the normal metabolic destruction of the catecholamines.[15]

If our working hypothesis concerning the relationship between the pressor response to cortically applied epinephrine and the catecholamine level of the hypothalamus is correct, animals pretreated with the amine oxidase inhibitor iproniazid should not show the usual cardiovascular reactions to cortically applied epinephrine. Our experiments have shown that rabbits pretreated with iproniazid presented an abolition, diminution, or reversal of the cardiovascular response to epinephrine applied on the frontoparietal area of the cerebral cortex.

TABLE 2
EFFECTS OF AMINE OXIDASE INHIBITORS ON CEREBRAL CORTICAL PRESSOR RESPONSE TO EPINEPHRINE

Drug	Dose mg./kg.	Number of animals	Pressor response after third application of epinephrine (mm. Hg ± S. E.*)	P
Serine N-isopropylhydrazide (RO 4-1038)	100	6	13 ± 4	0.001
DL-1-(N-acetylmethionyl)-2-isopropylhydrazine (RO 4-1018)	100	8	19 ± 4	0.001
Iproniazid (Marsilid)	50	4	32 ± 7	—
	100	19	16 ± 3	0.001
	300	3	7 ± 2	—
Isoniazid	100	4	51 ± 7	—
Control	—	16	53 ± 5	—

* S.E. = standard error.

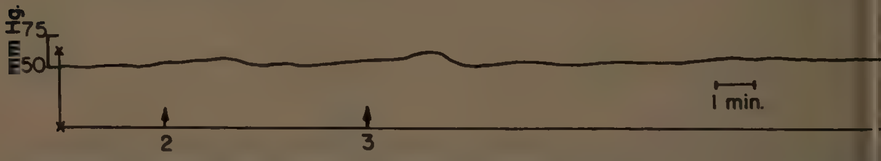

FIGURE 2. Rabbit blood pressure. Effect of cortically applied epinephrine in an animal pretreated 16 hours previously with iproniazid (100 mg./kg.).

In addition to iproniazid, we had the opportunity to test 2 other amine oxidase inhibitors, namely, serine N-isopropylhydrazide (RO 4-1038) and DL-1-(N-acetylmethionyl)-2-isopropylhydrazine (RO 4-1018).

Rabbits were injected with the amine oxidase inhibitors subcutaneously and tested 5 and 16 hours later with the cortically applied epinephrine. The cortical applications were at 10-min. intervals. TABLE 2 summarizes these findings.

All animals pretreated with systemically administered iproniazid and the other amine oxidase inhibitors showed a diminution of the pressor response to the first 6 or 8 applications of the cortically applied epinephrine (FIGURE 2).

In some experiments subsequent applications elicited increased pressor responses until an essentially normal response was obtained. Often the animals died at the time of the eighth to twelfth application; this was not seen in the control animals. Animals tested 16 hours after the administration of the amine oxidase inhibitors consistently showed the greatest diminution of the cortically applied epinephrine pressor response. Eight animals not listed in TABLE 2 showed a small depressor response to the topical application of epinephrine on the cerebral cortex (FIGURE 1). This depressor response sometimes coincided with the epinephrine application, but it often appeared 1 to 2 min. after the filter paper was removed.

In experiments in which systemic premedication with iproniazid had effectively inhibited the cortical reaction to epinephrine, a subsequent intravenous administration of reserpine (1 mg./kg.) led to the reappearance and to a gradually progressive increase of the cortically induced pressor effect 2 hours later (FIGURE 3). Moreover, in rabbits pretreated with reserpine and reacting with the usual augmentation of the cortical epinephrine pressor response, a subsequent intravenous administration of iproniazid (100 mg./kg.) produced a considerable decrease of this reaction. The hypertensive effect which, under the influence of premedication with reserpine, had reached values of 110 to 120 mm. Hg dropped to values of 30 to 50 mm. Hg 2 hours after the administration of iproniazid (FIGURE 4). This decrease is particularly characteristic, for the sensitizing action of reserpine on cortical responses to epinephrine generally persists unchanged during a period of 72 hours after the administration of that drug.

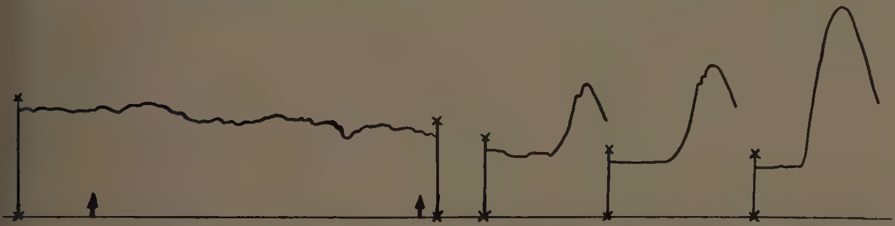

FIGURE 3. Rabbit blood pressure. Antagonism of the iproniazid effect on the cortical epinephrine pressor response by reserpine. At the first arrow, third application of epinephrine on the cortex of an animal pretreated with iproniazid, 100 mg./kg. 16 hours previously; at the second arrow, reserpine (1 mg./kg.). After 2 hours, 3 successive applications of epinephrine to the cerebral cortex produce essentially normal pressor responses.

Discussion

The striking antagonism between the central effects of reserpine and iproniazid in our experiments points to the possible existence of a close dependency of the cardiovascular reaction to cortically applied epinephrine upon variations of the catecholamine content of the hypothalamus. When this content is normal, normal pressor responses are obtained. When the content of catecholamines in the brain is lowered, as with reserpine, there is an exaggerated cardiovascular response. When the content is raised, as with iproniazid or the other amine oxidase inhibitors, there is an inhibition of this pressor response.

Among other items of evidence along these lines is the fact that animals pretreated with tetrabenazine (RO 1-9569, 40 mg./kg.) show an augmented pressor effect to the cortically applied epinephrine. Tetrabenazine, a sedative drug similar in pharmacological action to reserpine, has been shown to cause a decrease in the catecholamine content of the brain.[16] If 3,4,dihydroxy phenylalanine (L-DOPA), the precursor of the catecholamines, was systemically administered (100 mg./kg.), the cortical epinephrine pressor response was abolished 3 hours later. The catecholamine content of rabbit brain can be raised by systemic premedication with L-DOPA.

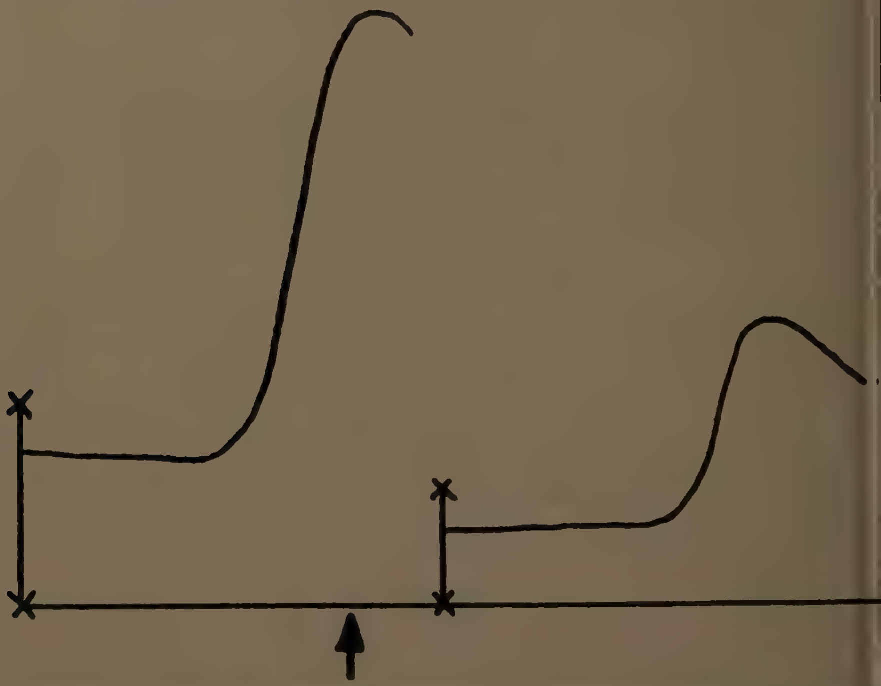

FIGURE 4. Rabbit blood pressure. Antagonism of the reserpine effect on the cortical epinephrine pressor response by iproniazid. (*Left*) the third application of epinephrine on the cortex of an animal pretreated with reserpine (1 mg./kg.) 4 hours previously; (*right*) 2 hours after the administration of iproniazid (100 mg./kg.): the third application of epinephrine to the cerebral cortex is significantly diminished.

It is interesting to note that drugs that influence catecholamine content of brain are capable of interfering with the well-balanced steady-state condition that apparently regulates the reactivity of cortical effectors to epinephrine. It would seem that, with an increased catecholamine content, there would be a saturation of the effector sites so that the application of epinephrine to the cortex of such an animal would have no effect. This saturation of effector sites may be the reason that the cortical epinephrine pressor effect was blocked with a massive dose (750 μg.) of epinephrine administered intravenously. Conversely, the reduction of catecholamine content in the brain would lead to a greater number of unoccupied effector sites, which may account for the augmentation of the cortical epinephrine pressor response.

Another item of interest is the fact that the amine oxidase inhibitors have the same effect on the pressor response of topically applied epinephrine as does schizophrenic serum. The catecholamine content of the brain is raised with the amine oxidase inhibitors and with schizophrenic serum. The effect of the amine oxidase inhibitors may, indeed, involve receptor sites that are particularly sensitive to the action of the unknown agent or agents contained in serum from schizophrenic patients.

Summary

(1) The amine oxidase inhibitors diminish the pressor response to epinephrine applied topically to the cerebral cortex of rabbits.

(2) Reserpine and tetrabenazine augment the pressor response to epinephrine applied topically.

(3) Under certain conditions iproniazid can antagonize the effects of reserpine on the cortically applied epinephrine pressor response.

(4) The mechanism of the inhibition to cortically applied epinephrine is concerned with the catecholamine content of the hypothalamus since, when the content is normal, normal responses are obtained. When the content is lowered, as with reserpine or tetrabenazine, there is an exaggerated cardiovascular response. When the content is raised, as with the amine oxidase inhibitors, schizophrenic serum, or L-DOPA, there is an inhibition of the cardiovascular response.

Acknowledgments

We are indebted to Alfred Pletscher, Director of Medical Research, F. Hoffmann-La Roche & Co., Ltd., Basel, Switzerland, for the gift of Marsilid, RO 4-1018, RO 4-1038, and tetrabenazine. We are also indebted to G. Zubowicz, Superintendent of Osawatomie State Hospital, who instituted the creation of a schizophrenia research ward.

References

1. Minz, B. & E. Domino. 1952. Effects of epinephrine and nor-epinephrine on electrically induced seizures. J. Pharmacol. Exptl. Therap. **107:** 204.
2. Minz, B. & E. King. 1952. Epinephrine modification of the effects of brain stem stimulation on the patellar and linguomandibilar reflexes. J. Pharmacol. Exptl. Therap. **106:** 407.
3. Minz, B., A. Remond & W. S. McCulloch. 1953. Local effects of epinephrine on the cerebral cortex. Proc. Soc. Exptl. Biol. Med. **83:** 306.
4. Lindemann, E. 1934. The psychopathological effect of drugs affecting the vegetative system. Am. J. Psychiat. **91:** 983.
5. Funkenstein, D. H. 1956. Nor-epinephrine and epinephrine-like substances in relation to human behavior. J. Nervous Mental Disease. **124:** 58.
6. Hoffer, A., H. Osmond & J. Smythies. 1954. Schizophrenia: a new approach II. J. Mental Sci. **100:** 29.
7. Chamorro, A. & B. Minz. 1956. Sensibilisation à distance par l'adrénaline appliquée sur le cortex cérébral du lapin. Compt. rend. soc. biol. **150:** 299.
8. Chamorro, A. & B. Minz. 1955. Réaction hypothalamique a une stimulation humorale du cortex cérébral. Compt. rend. soc. biol. **149:** 309.
9. Minz, B. 1957. Actions de drogues tranquillisantes sur la réaction du cortex cérébral a l'adrénaline. Compt. rend. soc. biol. **151:** 432.
10. Minz, B. & E. J. Walaszek. 1957. Sur la présence de substances biologiquement actives dans le sérum de schizophrènes. Compt. rend. **244:** 1974.
11. Minz, B. & E. J. Walaszek. 1958. Topic action of epinephrine on the cerebral cortex

of rabbits pretreated with serum from schizophrenics. J. Pharmacol. Exptl. Therap. **122:** 53A.

12. Walaszek, E. J., C. M. Smith & B. Minz. 1958. Effect of serum from schizophrenic patients on neurohumors in rabbit brain. Federation Proc. **17:** 416.
13. Carlsson, A., E. Rosengren, A. Bertler & J. Nilsson. 1957. Effect of reserpine on the metabolism of catecholamines. International Symposium on Psychotropic Drugs. Milan, Italy. : 363.
14. Spector, S., D. Prockop, P. A. Shore & B. B. Brodie. 1958. Effect of iproniazid on brain levels of nor-epinephrine and serotonin. Science. **127:** 704.
15. Shore, P. A., J. A. R. Mead, R. G. Kuntzman, S. Spector & B. B. Brodie. 1957. On the physiologic significance of monoamine oxidase in brain. Science. **126:** 1063.
16. Pletscher, A., H. Besendorf & H. P. Bächtold. 1958. Benzochinolizine, eine neue Korperklasse mit Wirkung auf den 5-Hydroxytryptamin und Noradrenalin Stoffwechsel des Gehirns. Arch. exptl. Pathol. u. Pharmakol. Naunyn-Schmiedeberg's. **232:** 499.

Discussion of the Paper

Julius Axelrod (*Laboratory of Clinical Science, National Institute of Mental Health, Public Health Service, Bethesda, Md.*): A number of papers presented in this monograph imply that monoamine oxidase is the enzyme chiefly concerned with the metabolism of epinephrine and norepinephrine. Recent investigations have shown that the inactivation of monoamine oxidase by iproniazid (Marsilid*) did not prolong the physiological action of the catecholamines in man or other mammals, nor did it alter the excretion of these compounds appreciably.[1-3] These observations suggested that epinephrine and norepinephrine were metabolized by pathways other than deamination. Studies in our laboratory have demonstrated the O-methylation of catecholamines in the organism as well as the normal occurrence of metanephrine (3-O-methylepinephrine) and normetanephrine (3-O-methylnorepinephrine) in urine and tissues.[4, 5] An enzyme that O-methylates catecholamines has also been found.[4]

The extent to which epinephrine is O-methylated and deaminated was examined in rats[6] and man,[7] using β-H^3-epinephrine. After the intraperitoneal injection of β-H^3-epinephrine to rats, about 55 per cent of the radioactivity was present in the urine as free and conjugated metanephrine, 12 per cent as 3-methoxy-4-hydroxymandelic acid, and only traces as 3,4-dihydroxymandelic acid. The administration of metanephrine resulted in the excretion of essentially the same amount of metanephrine (free and conjugated) and 3-methoxy-4-hydroxymandelic acid as was found after epinephrine.

To delineate further the role of monoamine oxidase in the metabolism of epinephrine, the effect of a monoamine oxidase inhibitor was examined. When rats were pretreated with iproniazid, almost all of the administered epinephrine or metanephrine was excreted as free and conjugated metanephrine. In addition, the excretion of 3-methoxy-4-hydroxymandelic acid was markedly reduced. These results indicate that monoamine oxidase is primarily concerned with the deamination of metanephrine in the rat.

After the administration of norepinephrine to the rat, large amounts of free and conjugated normetanephrine were excreted.[8] Pretreatment with iproniazid led to a marked elevation in the excretion of normetanephrine.

Large amounts of normetanephrine were found in the urine of patients with pheochromocytomas,[9] suggesting that O-methylation of catecholamines also occurs in man. After the intravenous infusion of β-H^3-epinephrine to man about 55 per cent of the radioactivity was present in the urine as free and conjugated metanephrine and 35 per cent as 3-methoxy-4-hydroxymandelic acid. Less than 3 per cent of the radioactivity found in the urine was 3,4-dihydroxymandelic acid. When metanephrine was administered, about the same fraction was excreted as metanephrine (free and conjugated) and 3-methoxy-4-hydroxymandelic acid as was observed after H^3-epinephrine.

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* Hoffmann-La Roche.

These findings demonstrate that O-methylation is the principal pathway for the metabolism of epinephrine and norepinephrine, and that monoamine oxidase is concerned mainly with the deamination of the O-methylated metabolites.

It appears highly likely that O-methylation also constitutes an important pathway for the metabolism of endogenous catecholamines in the nervous system. Normetanephrine has been found to be present in the brain[10] and in tissues rich in sympathetic fibers, for example, spleen and adrenal gland.[5] Furthermore, catechol-O-methyl transferase, the enzyme that catalyzes O-methylation, is present in all areas of the brain as well as the sympathetic nervous system.[11]

References

1. Griesemer, E. C., J. Barsky, C. A. Dragstedt, J. A. Wells & E. A. Zeller. 1953. Potentiating effects of iproniazid on the pharmacological action of sympathomimetic amines. Proc. Soc. Exptl. Biol. Med. **84:** 699.
2. Corne, S. J. & J. D. Graham. 1957. The effect of inhibition of amine oxidase *in vivo* on administered adrenaline, noradrenaline, tyramine and serotonin. J. Physiol. **135:** 339.
3. Friend, D. G., M. S. Zileli, J. T. Hamlin & F. W. Reutter. 1958. The effect of iproniazid on the inactivation of norepinephrine in the human. J. Clin. Exptl. Psychopathol. **19:** 61.
4. Axelrod, J. 1957. O-methylation of epinephrine and other catechols *in vitro* and *in vivo*. Science. **126:** 400.
5. Axelrod, J., S. Senoh & B. Witkop. 1958. O-methylation of catechol amines *in vivo*. J. Biol. Chem. **233:** 697.
6. Axelrod, J. The metabolism of catechol amines *in vitro* and *in vivo*. Pharmacol. Revs. In press.
7. LaBrosse, E. A., J. Axelrod & S. S. Kety. 1958. O-methylation, the principal route of metabolism of epinephrine in man. Science. **128:** 593.
8. Axelrod, J., J. K. Inscoe, S. Senoh & B. Witkop. 1958. O-methylation, the principal pathway for the metabolism of epinephrine and norepinephrine in the rat. Biochim. et Biophys. Acta. **27:** 210.
9. LaBrosse, E. A., J. Axelrod & A. Sjoerdsma. 1958. Urinary excretion of normetanephrine in man. Federation Proc. **17:** 386.
10. Axelrod, J. 1958. Presence, formation and metabolism of normetanephrine in the brain. Science. **127:** 754.
11. Axelrod, J., R. W. Albers & C. Clementi. J. Neurochem. In press.

PHARMACOLOGY OF IPRONIAZID AND OTHER AMINE OXIDASE INHIBITORS

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Iproniazid (Marsilid) has been found to be a therapeutic agent with an unusually broad spectrum of activity.[1, 2] Initially introduced as an antitubercular drug, iproniazid has more recently been employed in the treatment of depressive states, angina pectoris, and hypertension. Iproniazid has been demonstrated to be a potent inhibitor of amine oxidase, an enzyme that has a role in the metabolism of serotonin, norepinephrine, and other biological amines and that prevents their accumulation in the body tissues.[3] Following the administration of iproniazid an increase in the levels of serotonin and norepinephrine occurs in the brain.[4-7] The precursor of serotonin, 5-hydroxytryptophan, and the precursor of catecholamines, 3,4-dihydroxyphenylalanine, both have excitatory effects, and this central excitatory activity can be potentiated by iproniazid.[8-10] However, when administered alone to animals, iproniazid causes little or no obvious central effects in nonlethal doses. These observations indicate that iproniazid mediates its central stimulating activity indirectly, probably by altering the metabolism of serotonin, of catecholamines and, possibly of other biological amines.

Iproniazid has the property of blocking many of the pharmacological effects of reserpine.[8, 9] Reserpine evokes central depression, a fall of brain serotonin and norepinephrine, ptosis, myosis, and a fall in body temperature. The effects induced by reserpine can be effectively blocked by iproniazid.[4-12]

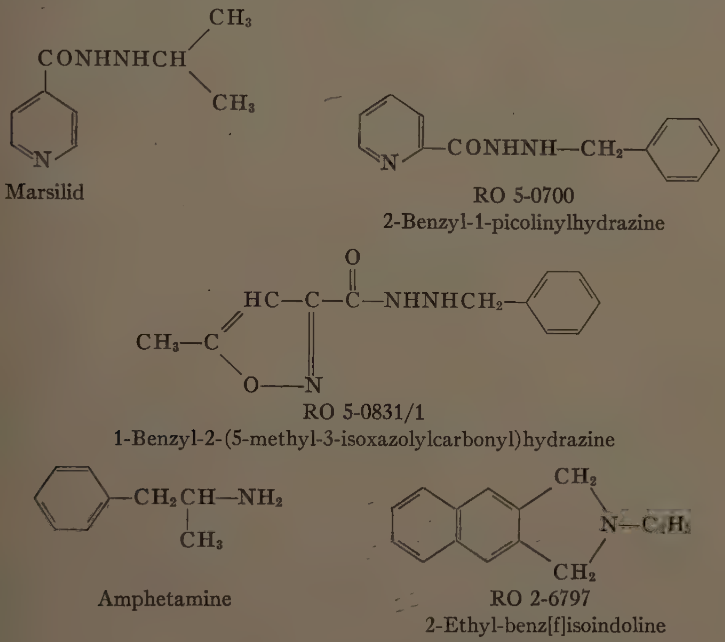
These observations were utilized in devising a series of pharmacological tests that have led to the development of 2 new compounds, RO 5-0700 and RO 5-0831/1, having much greater activity than iproniazid; the chemical structure of these highly potent agents is shown in FIGURE 1. Whereas iproniazid (RO 2-4572/3) contains the isopropylhydrazine moiety, RO 5-0700 and RO 5-0831/1 are derivatives of benzylhydrazine.

The purpose of this communication is to report information about the pharmacological activity of RO 5-0700 and RO 5-0831/1. For purposes of comparison and to extend knowledge of the mechanism whereby iproniazid, RO 5-0831/1, and RO 5-0700 exert their activity, data have also been obtained on amphetamine and on RO 2-6797/3, a benzisoindoline derivative. These agents are not derivatives of hydrazine. Amphetamine and benzisoindoline both show direct central excitatory effects in animals in contrast to iproniazid, RO 5-0700, and RO 5-0831/1, which probably act indirectly by permitting an accumulation of biological amines in the tissues.

Methods

Inhibition of amine oxidase *in vitro* was determined by the manometric technique.[13] Rats were sacrificed, the livers rapidly excised, and 10 per cent homogenates prepared with isotonic (0.25 M) sucrose. Differential centrifugation was then performed. One ml. of a liver mitochondrial suspension in

Sorenson's phosphate buffer (*p*H 7.2), equivalent to 300 mg. of liver per ml., served as the source of enzyme activity. Tyramine, in a final concentration of 0.01 M, was employed as the substrate, and a final concentration of 0.0033 M sodium cyanide was added to restrict activity to that of monoamine oxidase; the final volume was adjusted to 3.0 ml. with buffer solution. The center well contains 0.2 ml. of 2 M KOH to absorb CO_2. After gassing for 5 min. with 100 per cent O_2, the flasks were incubated for 20 min. in the Warburg bath at 38° C. The substrate contained in the side arm was then tipped into the main compartment. Oxygen uptake was determined every 10 min. for 30 min.


Marsilid

RO 5-0700
2-Benzyl-1-picolinylhydrazine

RO 5-0831/1
1-Benzyl-2-(5-methyl-3-isoxazolylcarbonyl)hydrazine

Amphetamine

RO 2-6797
2-Ethyl-benz[f]isoindoline

FIGURE 1. Structural formulas.

In vivo amine oxidase inhibitory activity was determined by the method described by Davison.[13] Rats were given various doses of the compounds intraperitoneally and were sacrificed 1 hour after administration. For these measurements, 4 rats were employed at each dosage level. The brains were rapidly removed, a 10 per cent homogenate in distilled water was prepared, and the particulate fraction was separated by centrifugation. The particulate matter was resuspended in Sorenson's 0.67 M phosphate buffer, (*p*H 7.2) so that 1 ml. contained the equivalent of 400 mg. of wet brain tissue. The test system consisted of 0.2 ml. of 0.032 M potassium cyanide and 0.2 ml. of 0.16 M tyramine in the side arm, while 1.0 ml. of the particulate suspension and 1.6 ml. of *p*H 7.2 phosphate buffer was added to the main compartment. The center well contained 0.2 ml. of KOH. After gassing with 100 per cent O_2 and equilibrating at 38° C., the contents of the side arm were tipped in, and readings were taken at 10 min. intervals for 30 min. Results were expressed as microliters O_2/gm. of brain tissue per hour and calculated as percentages of inhibition of control values.

The blocking of reserpine ptosis was demonstrated by giving reserpine 0.75 mg./kg. subcutaneously to rats at various intervals after the administration of iproniazid or an analogue and observing for ptosis at 4 hours.

Iproniazid will potentiate the excitatory activity of 5-hydroxytryptophan and dihydroxyphenylalanine in mice.[8-10] In the present experiments mice were premedicated with various intraperitoneal dosages of the compounds to be tested 1 hour before the intraperitoneal administration of 100 mg./kg. of 5-hydroxytryptophan or 3,4-dihydroxyphenylalanine. The dose causing excitation in 50 per cent of the mice was determined. In this manner the relative potency of RO 5-0700 and RO 5-0831/1 in comparison with iproniazid was determined. In addition, the rectal temperatures of these mice were taken with an electronic thermometer, and the doses of iproniazid, RO 5-0700, and RO 5-0831/1 which caused a rise of 1° C. were calculated from the log dose response curves.

TABLE 1

IN VITRO AND *IN VIVO* INHIBITION OF AMINE OXIDASE

Drug	*In vitro* molar concentration producing 50 per cent inhibition of amine oxidase	Potency ratio	ED_{50} *in vivo* mg./kg.	Potency ratio
Iproniazid	5.3×10^{-6}	1	25	1
RO 5-0700	7.7×10^{-7}	7	1	25
RO 5-0831/1	8×10^{-7}	7	0.75	33
Amphetamine	9×10^{-4}	1/200	>50	0
Benzisoindoline	1×10^{-4}	1/20	>50	0

Experimental

Amine oxidase inhibition. The molar concentrations of the compounds producing 50 per cent inhibition of liver amine oxidase activity *in vitro* and the relative potency in comparison with iproniazid are shown in TABLE 1. From these measurements, it was found that the *in vitro* inhibitory activity of RO 5-0831/1 and RO 5-0700 is sevenfold more potent than that of iproniazid, while amphetamine and benzisoindoline are weak inhibitors of amine oxidase *in vitro*.

The inhibition of brain amine oxidase activity *in vivo* exerted by RO 5-0700 and RO 5-0831/1 was found to be considerably higher than that of iproniazid. The ED_{50} of iproniazid was calculated to be 25 mg./kg., while the ED_{50} of RO 5-0700 is 1.0 mg./kg. and that of RO 5-0831/1 is 0.75 mg./kg. Thus, the potency of RO 5-0700 is twenty-fivefold that of iproniazid and RO 5-0831/1 exhibits a thirty-threefold greater activity. In contrast, amphetamine and benzisoindoline do not inhibit amine oxidase activity of rat brain *in vivo* even when given at toxic doses.

The long duration of amine oxidase inhibitory activity produced by iproniazid and the benzylhydrazine analogues is shown in FIGURE 2. In these experiments, groups of rats were administered the indicated doses of each compound and then were sacrificed at various time intervals over a 25-day period. Each time point represents the average of measurements performed on 4 animals. RO 5-0700 and RO 5-0831/1 are at least 25 times more potent than iproniazid as inhibitors of brain amine oxidase, and their duration of action is equally prolonged at the effective dose levels of each compound.

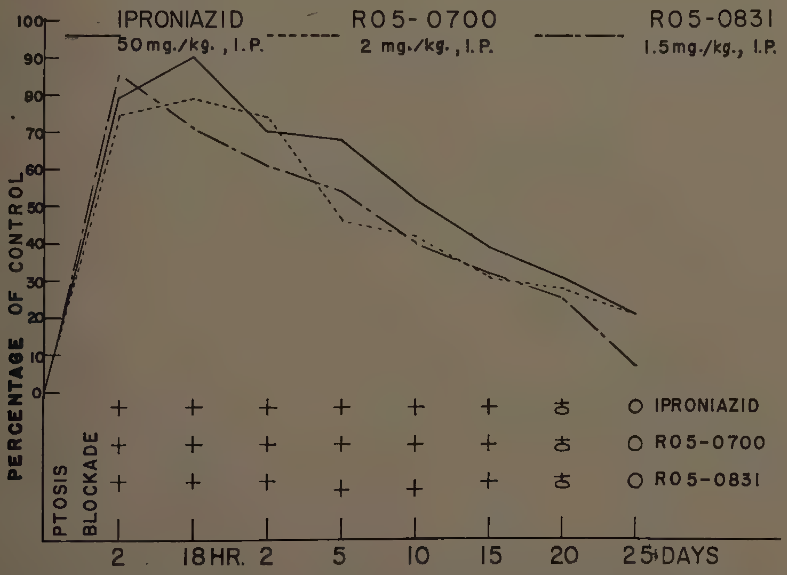


FIGURE 2. The duration of amine oxidase inhibition of rat brain *in vivo* by iproniazid (50 mg./kg. intraperitoneally), RO 5-0700 (2 mg./kg.), and RO 5-0831 (2 mg./kg.). The ptosis induced by reserpine (0.75 mg./kg.) was measured at various hours after iproniazid was introduced as an analogue.

5-Hydroxytryptophan (5-HT) and 3,4-Dihydroxyphenylalanine (DOPA) Potentiation

Potentiation of the central stimulation and rise in body temperature induced in mice by 5-HTP and DOPA was exhibited by iproniazid and the new analogues. RO 5-0700 and RO 5-0831/1 displayed tenfold to twentyfold higher potentiating activity than iproniazid in these tests (TABLE 2). An increase in rectal temperature regularly occurred at the same doses of iproniazid or its analogues that induced potentiation of DOPA. The higher potentiating activity of the benzylhydrazine analogues is in fair agreement with their potency as inhibitors of amine oxidase. However, iproniazid, RO 5-0700, and RO 5-0831/1 do not cause excitation in mice when administered alone in single nonlethal doses.

On the other hand, amphetamine and benzisoindoline produce direct excitation, salivation, and mydriasis in mice. Since they are direct stimulants to the nervous system, they are also capable of potentiating the responses of 5-HTP and DOPA. While the *in vitro* amine oxidase inhibitory activity exerted by amphetamine is one tenth that of benzisoindoline, it is much stronger in various pharmacological tests of central stimulating activity. Therefore, it appears unlikely that the central excitatory effects of amphetamine and benzisoindoline are related directly to inhibition of amine oxidase.

Influence on Responses to Reserpine in Mice

Reserpine produces sedation and ptosis in mice, and these responses to reserpine can be blocked by prior administration of iproniazid.[8, 9] However, direct central stimulants of the amphetamine class are also capable of blocking the sedation and ptosis induced by reserpine and can also effect a reversal of these responses to reserpine after they have been elicited. Amphetamine and benzisoindoline, when administered alone to mice, produce an increase in motor activity and central excitation, salivation, and mydriasis. These signs of central stimulatory and sympathomimetic activity are not at all present with the amine oxidase inhibitors iproniazid, RO 5-0700, and RO 5-0831/1.

TABLE 2

POTENTIATION OF 5-HYDROXYTRYPTOPHAN (5-HT) AND 3,4-DIHYDROXYPHENYLALANINE (DOPA) RESPONSES IN COMPARISON WITH IPRONIAZID

Drug	Amine oxidase inhibition *in vitro* potency	Direct excitation potency	5-HTP potentiation	DOPA potentiation
Iproniazid	1	0	1	1
RO 5-0700	7	0	10	10
RO 5-0831/1	7	0	20	20
Amphetamine	1/200	1	50	100
Benzisoindoline	1/20	1/6	10	4

TABLE 3

EFFECT OF CENTRAL EXCITATORY AGENTS ON RESERPINE-INDUCED PTOSIS IN MICE

Drug	Direct excitation potency	Reserpine ptosis	
		Prevention potency	Reversal potency
Iproniazid	0	1	0
RO 5-0700	0	6	0
RO 5-0831/1	0	7	0
Amphetamine	1	1/3	1
Benzisoindoline	1/6	3/4	1/4

These observations may serve as the basis for a test that differentiates direct central stimulants of the amphetamine type from indirect central stimulants, such as the hydrazides. The results of experiments of this nature are shown in TABLE 3.

These results reveal that the hydrazides iproniazid, RO 5-0700, and RO 5-0831/1 can prevent reserpine-induced ptosis, but cannot reverse the effects of reserpine once they have been elicited. Also, the amine oxidase inhibitors do not exhibit direct central stimulating activity in mice. Therefore, it appears that inhibitors of amine oxidase exert their activity in a qualitatively different manner than do other central stimulants.

The extremely long duration of action of iproniazid, RO 5-0700, and RO 5-0831/1 in blocking reserpine effects are reconstructed in FIGURE 2. The amine oxidase inhibition of rat brain *in vivo* and the blocking of reserpine ptosis in rats can be demonstrated for about 20 days. These results confirm the long duration of iproniazid action demonstrated by Zeller *et al.*[14]

Toxicology

Single administration. The acute toxicity of iproniazid, RO 5-0700, and RO 5-0831/1 was investigated by the administration of single doses to mice, rats, dogs, and monkeys. These data are shown in TABLE 4.

TABLE 4
ACUTE TOXICITY OF IPRONIAZID, RO 5-0700 AND RO 5-0831/1

Species	Route	Iproniazid mg./kg.	RO 5-0700 mg./kg.	RO 5-0831/1 mg./kg.
Mice	P.O.	959 ± 29	*LD_{50} ± S.E.* 448 ± 128	193 ± 5
Mice	I.P.	690	187 ± 12	190 ± 18
Rat	P.O.	383	*LD_{50}* 240	280
Dog	P.O.	95	*Lethal dose* 95	>40
Monkey	P.O.	640	*Lethal dose* 240	160

From these data, iproniazid was observed to be less toxic than RO 5-0831/1 and RO 5-0700 when given in single doses to mice and monkeys, while the lethal doses of the 3 drugs for rats and dogs were more nearly comparable. Administration of oral doses of RO 5-0831/1 higher than 40 mg./kg. to dogs was prevented by the occurrence of emesis. Iproniazid and RO 5-0700 were more toxic to mice by the intraperitoneal route than when given orally, while the LD_{50} values of RO 5-0831/1 by both routes are equal. This finding suggests that RO 5-0831/1 is readily absorbed from the gastrointestinal tract following oral administration. The LD_{50} in all species tested is considerably above the clinical doses required to effect a therapeutic response.

Repeated administration. Monkeys tolerated a total oral dose of 20 mg. of RO 5-0700 and RO 5-0831/1 daily for 14 days without toxic manifestations.

Groups of rabbits were administered 5 mg./kg., 10 mg./kg. and 15 mg./kg. of RO 5-0700 and RO 5-0831/1 by the subcutaneous route daily for 30 days. At the 15 mg./kg. dose of RO 5-0700, a moderate leukopenia and rise in segmented neutrophils was observed. At the highest dose of RO 5-0700, 1 rabbit expired on the twenty-seventh experimental day. One rabbit receiving 10 mg./kg. of RO 5-0831/1 succumbed on the twenty-fifth day, but the hematological findings in the group of rabbits given RO 5-0831/1 remained within normal limits.

Dogs given 5 mg./kg, of RO 5-0700 and RO 5-0831/1 orally for 30 days remained free of toxic manifestations. The treated animals displayed weight gains comparable to those of controls, and no reduction in hematocrit, hemoglobin, leukocyte, or differential leukocyte counts was observed. Five mg./kg. of iproniazid administered to dogs for a similar period is tolerated without signs of toxicity.

Thirteen-week chronic toxicity studies of iproniazid, RO 5-0700, and RO 5-0831/1 were conducted in rats. The doses used of each amine oxidase inhibitor were 2.5 mg./kg., 5.0 mg./kg., and 10 mg./kg. of body weight and were administered as an admixture in the diet. Similar experiments were conducted with benzisoindoline at doses of 10 mg./kg., 20 mg./kg., and 40 mg./kg. Hematological values were obtained at the beginning and at the fourth, eighth, and twelfth experimental weeks. These results were compared with data obtained in rats using iproniazid dihydrochloride in approximate dosages of 5 mg./kg., 20 mg./kg., and 80 mg./kg.[15] At each dose level, 5 males and 5 females were used; the growth and hematological data represent the average of 10 animals.

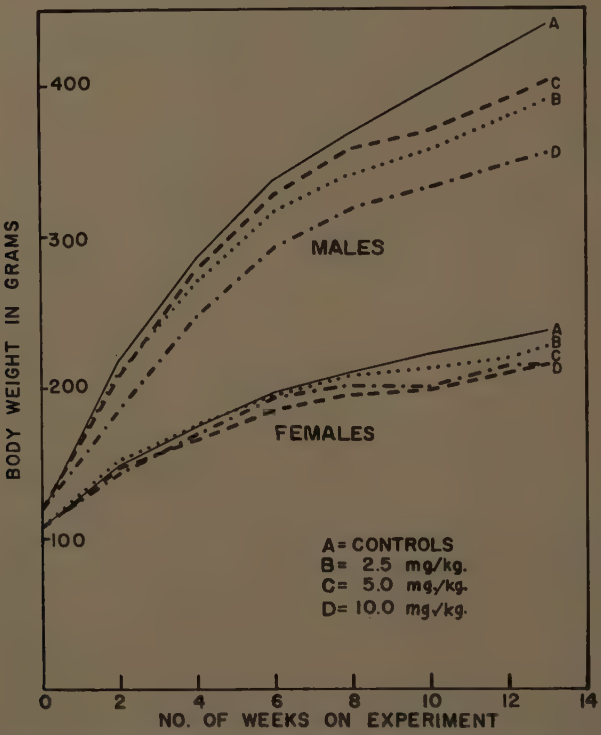


FIGURE 3. Growth curves of rats in a 13-week chronic toxicity study of the effects of RO 5-0700 in the diet.

From the growth response curves shown in FIGURES 3, 4, 5, and 6 some depression in growth in rats given 10 mg./kg. of RO 5-0700 and RO 5-0831/1 was observed. Rats administered 10 mg./kg. of iproniazid exhibited growth comparable to that of the control group. Results of earlier studies revealed that a dose of 20 mg./kg. of the dihydrochloride salt of iproniazid produced a depression in growth.[15]

The results of hematological studies of iproniazid, RO 5-0700, RO 5-0831/1, and RO 2-6797/3 revealed no alterations in hematopoietic tissues. At 20 mg./kg. and 80 mg./kg., iproniazid was previously found to produce a moderate decrease in red cell and leukocyte counts and in hemoglobin values.[15]

The results of toxicity measurements in a number of species indicate that the much higher potency of RO 5-0700 and RO 5-0831/1 over iproniazid as inhibitors of amine oxidase is not associated with a concomitant higher toxicity. This suggests that lower dosages of RO 5-0700 and RO 5-0831/1 than of iproniazid could be employed clinically in order to achieve a therapeutic response, thus obviating some of the undesirable side effects.

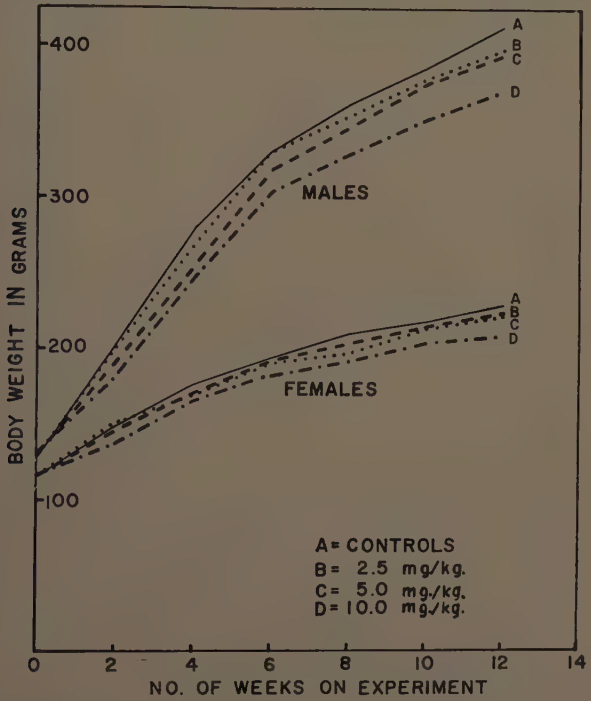


FIGURE 4. Growths curves of rats in a 13-week chronic toxicity study of the effects of RO 5-0831/1 in the diet.

Discussion

The present investigation was conducted to obtain pharmacological and toxicological information about 2 benzylhydrazine analogues of iproniazid. These compounds, RO 5-0700 and RO 5-0831/1, were found to be considerably more potent as *in vitro* and *in vivo* inhibitors of amine oxidase. They also were capable of greatly potentiating the central stimulatory activity of the precursors of serotonin and catecholamines in intact mice. These findings suggest that the administration of RO 5-0700 and RO 5-0831/1 may cause the accumulation of serotonin and catecholamines in the brain in a manner similar to iproniazid[4, 5]; therefore, the benzylhydrazine analogues may also act as psychic energizers. Comparative studies were performed using the direct central stimulants amphetamine and a new drug, benzisoindoline. The results obtained demonstrate that RO 5-0700, RO 5-0831/1, and iproniazid exhibit pharmacological properties that are qualitatively different from those of sympathomimetic amines that exert central stimulatory activity.

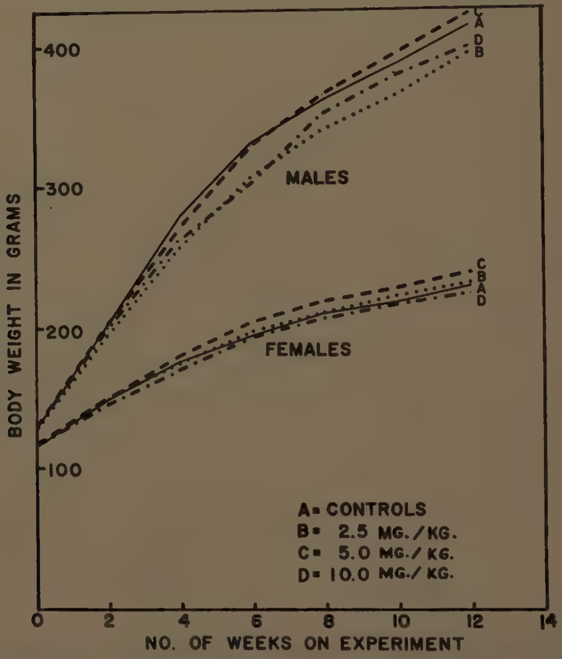


FIGURE 5. Growth curves of rats in a 13-week chronic toxicity study of the effects of iproniazid in the diet.

Toxicological studies of these agents were conducted in mice, rats, dogs, and monkeys. Despite the considerably higher potency of RO 5-0831/1 and RO 5-0700 as inhibitors of amine oxidase, they were found not to exert a concomitant higher toxicity. Whereas the group of rats given 10 mg./kg. of iproniazid for 13 weeks did not differ from controls, some depression in growth was observed at this dose of RO 5-0831/1 and RO 5-0700. Hematologic findings were within normal limits. In experiments reported previously with iproniazid dihydrochloride, a depression in growth accompanied by a decrease in erythrocytes, leukocytes, and hemoglobin was observed in rats given dietary doses equivalent to 20 mg./kg. and 80 mg./kg.[15] In all of these toxicological studies the doses employed were considerably higher than the recommended clinical doses.

Summary

RO 5-0700 (2-benzyl-1-picolinylhydrazine) and RO 5-0831/1 (1-benzyl-2-[5-methyl-3-isoxazolylcarbonyl] hydrazine) are benzyl hydrazine analogues of iproniazid (Marsilid) that are much stronger amine oxidase inhibitors than iproniazid. This effect was demonstrated on amine oxidase of rat liver mitochondria *in vitro* and on rat brain mitochondria *in vivo*. Like iproniazid, the duration of action *in vivo* is 20 days after a single dose of each.

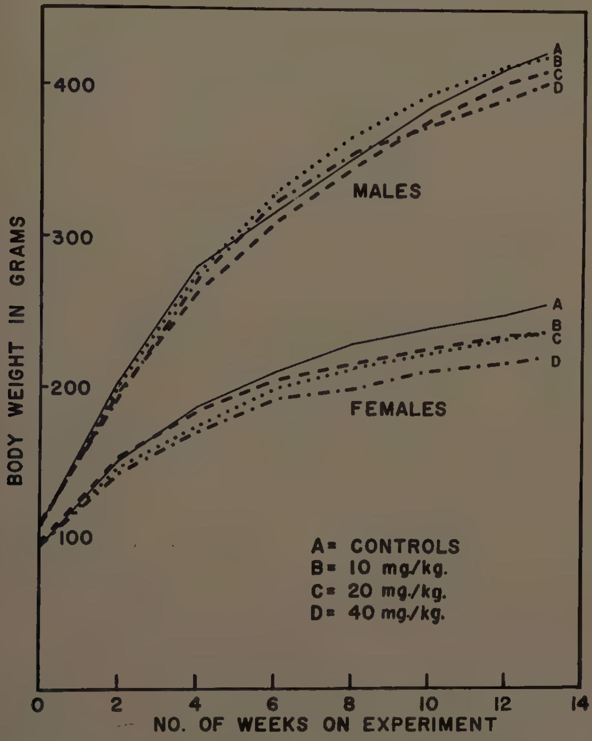


FIGURE 6. Growth curves of rats in a 13-week chronic toxicity study of the effects of RO 2-6797/3 in the diet.

These compounds potentiate the central excitatory activity of 5-HTP (5-hydroxytryptophan) and of DOPA (3,4-dihydroxyphenylalanine), but do not directly excite the animals. They prevent the sedative effects of reserpine, but do not reverse its effects.

On the other hand, RO 2-6797/3 (2-ethyl-benz[f]isoindoline phosphate) is a weak amine oxidase inhibitor which, like amphetamine, is a direct excitant to the central nervous system. It potentiates directly the central effects of 5-HTP and DOPA and reverses the depressant effects of reserpine.

Toxicological studies indicate that RO 5-0700 and RO 5-0831/1 at doses of 2.5, 5.0, and 10 mg./kg. in rats showed no effects on hematopoietic tissues. Growth curves were depressed at high doses of RO 5-0700 and RO 5-0831/1, but not by iproniazid. No toxicological effects were observed with RO 2-6797/3 at doses of 10, 20, and 40 mg./kg. On repeated administration, monkeys tolerated total daily oral doses of 20 mg. of RO 5-0700 and RO 5-0831/1 for 14 days, rabbits tolerated subcutaneous doses up to 10 mg./kg. for 30 days, and dogs tolerated 5 mg./kg. orally of RO 5-0700 and RO 5-0831/1 for 30 days.

Acknowledgment

The technical assistance of Beryl Kappell, Carol Callahan, Dorothy Hane, Harry Rudiger, Allen Nestle, Charles Impellizzeri, and Jean Courter is greatly appreciated.

The chronic toxicity experiments on RO 2-6797/3 were initiated by T. Wong.

References

1. BOSWORTH, D. M. 1958. Discussion of the history of Marsilid. J. Clin. Exptl. Psychopathol. **19**(1): 72.
2. KLINE, N. S. 1958. Clinical experience with iproniazid (Marsilid). J. Clin. Exptl. Psychopathol. **19**(1): 11.
3. ZELLER, E. A., J. BARSKY, J. R. FOUTS, W. F. KIRCHHEIMER & L. S. VAN ORDEN. 1952. Influence of isonicotinic acid hydrazide (INH) and 1-isonicotinyl-2-isopropyl hydrazide (IIH) on bacterial and mammalian enzymes. Experientia. **8**: 349.
4. UDENFRIEND, S., H. WEISSBACH & D. F. BOGDANSKI. 1957. Effect of iproniazid on serotonin metabolism *in vivo*. J. Pharmacol. Exptl. Therap. **120**: 255.
5. PLETSCHER, A. 1957. Wirkung von Isopropyl-isonicotinsäurehydrazid auf den Stoffwechsel von Catecholaminen und 5-Hydroxytryptamin im Gehirn. Schweiz. med. Wochschr. **78**: 1532.
6. BRODIE, B. B., J. S. OLIN, R. G. KUNTZMAN & P. A. SHORE. 1957. Possible interrelationship between release of brain norepinephrine and serotonin by reserpine. Science. **125**: 1293.
7. SHORE, P. A. 1958. Possible mechanism of antidepressant action of Marsilid. J. Clin. Exptl. Psychopathol. **19**(1): 56.
8. BOGDANSKI, D. F., H. WEISSBACH & S. UDENFRIEND. 1958. Pharmacological studies with the serotonin precursor, 5-hydroxytryptophan. J. Pharmacol. Exptl. Therap. **122**: 182.
9. CHESSIN, M., E. R. KRAMER & C. C. SCOTT. 1957. Modifications of the pharmacology of reserpine and serotonin by iproniazid. J. Pharmacol. Exptl. Therap. **119**: 453.
10. CARLSSON, A., M. LINDQVIST & T. MAGNUSSON. 1957. 3,4-Dihydroxyphenylalanine and 5-hydroxytryptophan as reserpine antagonists. Nature. **180**: 1200.
11. BÄCHTOLD, H. & A. PLETSCHER. 1957. Einfluss von Isonikotinsäurehydraziden auf den Verlauf der Körpertemperatur nach Reserpin, Monoaminen und Chlorpromazin. Experientia. **13**: 163.
12. HOLZBAUER, M. & M. VOGT. 1956. Depression by reserpine of the noradrenaline concentration in the hypothalamus of the cat. J. Neurochem. **1**: 8.
13. DAVISON, A. N. 1957. The Mechanism of irreversible inhibition of rat liver monoamine oxidase by iproniazid (Marsilid). Biochem. J. **67**: 312.
14. ZELLER, E. A., J. BARSKY & E. R. BERMAN. 1955. XI. Inhibition of monoamine oxidase by 1-isonicotinyl-2-isopropylhydrazine. J. Biol. Chem. **214**: 267.
15. BENSON, W. M., P. L. STEFKO & M. D. ROE. 1952. Pharmacologic and toxicologic observations on hydrazine derivatives of isonicotinic acid (Rimifon, Marsilid). Am. Rev. Tuberc. **65**: 376.

Discussion of the Paper

I. LEUSEN (*Laboratory of Physiopathology, University of Ghent, Ghent, Belgium*): In connection with the presentation of Randall and Bagdon, I propose to summarize the results obtained with iproniazid and some of its analogues in experiments in which the effect of these substances on the inotropic activity of the papillary muscle of the cat heart was examined.

Isopropyl isonicotinic acid hydrazide (iproniazid, Marsilid) seems to be of value in the treatment of angina pectoris, but the mechanism of this beneficial effect is not known. Previous studies have shown that iproniazid lowers the amplitude of the isolated perfused heart.[1, 2] This raises the question whether the action of the drug in angina pectoris is not due to a negative inotropic effect on the heart.

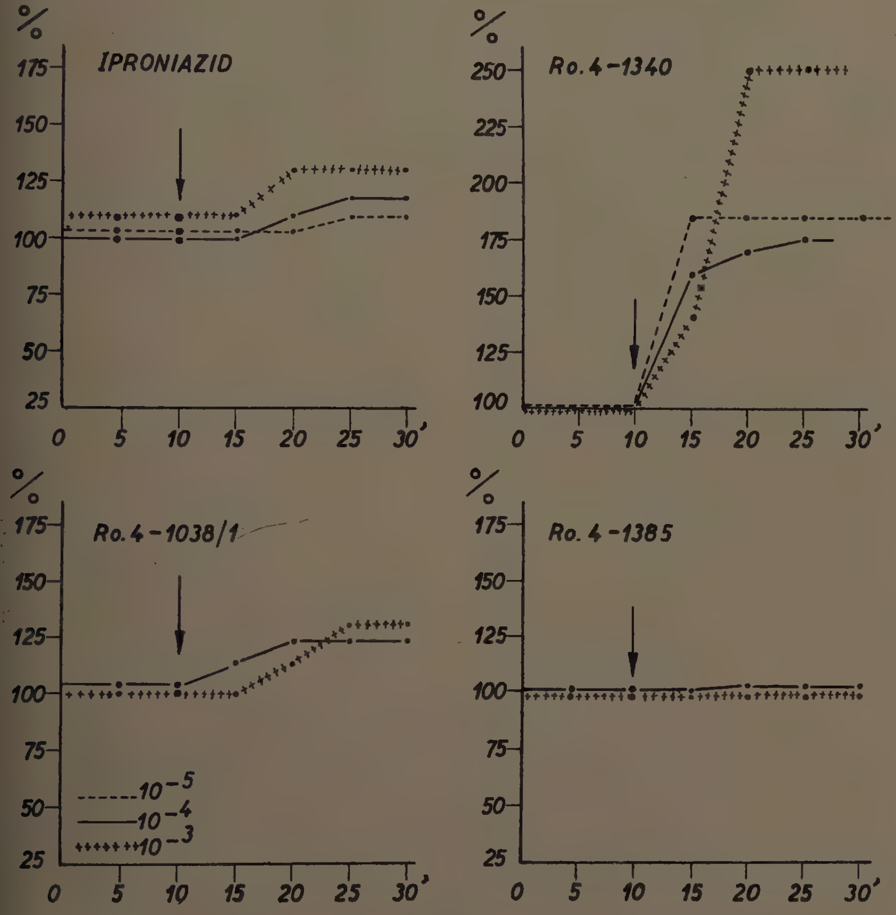


FIGURE 1

Methods. Each cat was etherized, the heart removed, and the papillary muscles isolated from the right side and mounted in a chamber filled with a modified Loche solution (NaCl, 130; $CaCl_2$, 2.65; glucose, 5.54; KCl, 4.69; $NaHCO_3$, 24 mmol./l.) through which an oxygen-CO_2 mixture (5 per cent CO_2) was passed. By means of a water bath and thermostat the temperature was kept at 37° C. The muscle was stimulated throughout the experiment at a rate of 60/min. by means of condenser discharges (0.75 microfarad, 45 volt). The contractions of the papillary muscle were recorded photographically according to a method described in detail elsewhere.[3] The principle of the method is that the contraction of the muscle causes the binding of a small steel spring attached to 4 strain gauges that in turn are connected in a Wheatstone bridge circuit. The binding of the spring changes the resistance of the strain gauge wires, producing a recorded deflection of a sensitive galvonometer.

After a control period lasting between 1½ to 2 hours, the amplitude of the contractions being well stabilized, a concentrated solution of iproniazid base, iproniazid phosphate, or one of the analogues [DL serin N^2 isopropropyl hydrazide·HCl (RO 4-1038/1), 1-α-alanine-isopropyl hydrazide·HCl (RO 4-1340) or L-glutaminic acid α-isopropyl hydrazide (RO 4-1385)] was added to the bath solution to make final concentrations of 10^{-5}, 10^{-4}, or 10^{-3}.

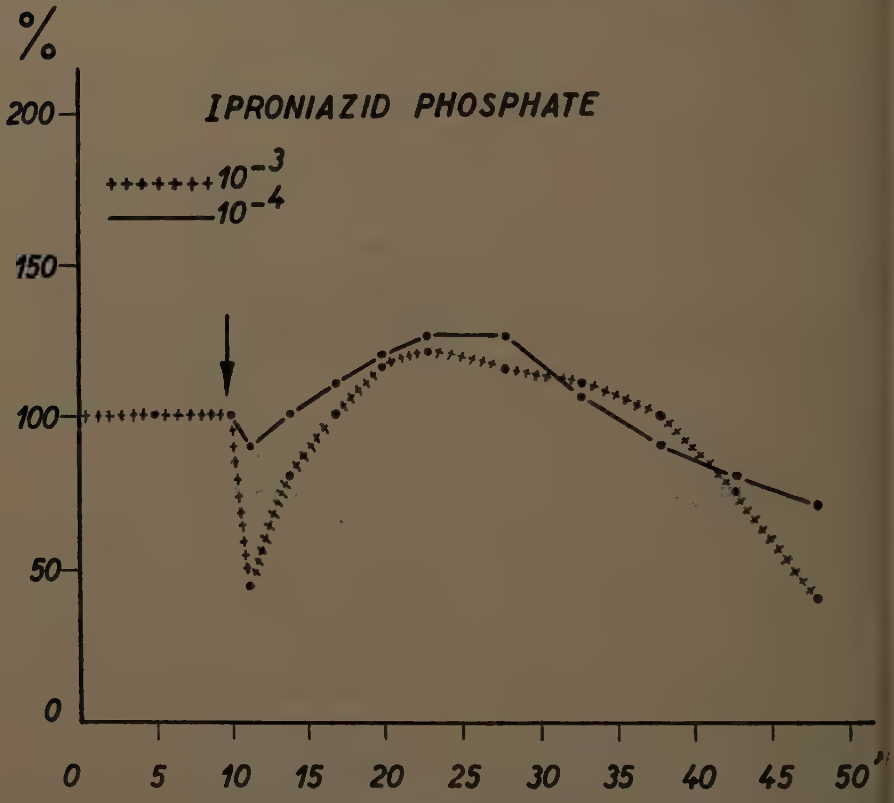


FIGURE 2

Results and discussion. Examples of the results obtained are plotted in FIGURE 1. It may be seen that iproniazid base at the final concentrations of 10^{-5} and 10^{-4} produces a small but constant positive inotropic effect on the papillary muscle. These results were uniform in all the experiments but, at a concentration of 10^{-3}, this positive effect was seen in most of the experiments, some preparations showing a slight depression of the amplitude.

On the other hand, iproniazid phosphate, at concentrations of 10^{-4} and 10^{-3}, showed completely different results. An initial short-lasting but sometimes profound depression of the amplitude was seen after the addition of the substance. This was followed by a more or less complete recovery and sometimes by a slight increase in the amplitude of the contractions. This phase was followed by a progressive depression and death of the muscle (FIGURE 2). At a concentration of 10^{-5} a slight positive inotropic effect of iproniazid phosphate was seen.

This difference between iproniazid base and its phosphate salt is probably due to the phosphate part of the molecule.

The serine derivative (RO 4-1038/1) showed effects comparable to iproniazid base. The glutamic acid derivative (RO 4-1385) at final concentrations of 10^{-4} and 10^{-3} had no clear effect on the inotropism of the papillary muscle. As shown by Zeller and his colleagues elsewhere in this monograph, this analogue probably has a much higher affinity for the brain and less for the heart than does iproniazid. On the other hand, the alanine derivative (RO 4-1340) had a very marked positive inotropic effect manifest at all concentrations used (10^{-5}, 10^{-4}, and 10^{-3}). In this respect this analogue was clearly different from the other substances tested, its effects resembling those of epinephrine. These results indicate that, apart from a different potency of inhibition of monoamine oxidase, the derivatives of iproniazid also can have quite different pharmacological effects on the inotropic activity of heart muscle.

Summary. Iproniazid base in concentrations of 10^{-5}, 10^{-4}, and 10^{-3} appears to have a slight positive inotropic effect on the isolated papillary muscle of the cat heart. Iproniazid phosphate at the higher concentrations depresses the muscle. Analogues of iproniazid showed different effects. The analogue L-α-alanine isopropyl hydrazide HCl (RO 4-1340) had a pronounced positive inotropic effect on the isolated muscle preparation.

References

1. ALLMARK, M. G., F. C. LU, E. CARMICHAEL & A. LAVALLEE. 1953. Some pharmacological observations on isoniazid and iproniazid. Am. Rev. Tuberc. **68:** 199.
2. PLETSCHER, A. & B. PELLMONT. 1958. Biochemical and pharmacologic actions of Marsilid on the heart. J. Clin. Exptl. Psychopathol. **19** (2) suppl. 1: 163.
3. LEUSEN, I., G. DEMEESTER & J. DE WITTE. 1955. Influence de l'acéthyl-digitoxine-α sur le muscle papillaire du chat. Arch. intern. pharmacodynamie. **102:** 153.

LEON I. GOLDBERG (*Section of Experimental Therapeutics, National Heart Institute, Public Health Service, Bethesda, Md.*): Most of the explanations of the actions of monoamine oxidase (MAO) inhibitors have been based on their effects on the metabolism of norepinephrine and serotonin. As other endogenous amines have now been described[1, 2] it appeared that these amines may also be important in explaining the actions of MAO inhibitors. Therefore, we studied the effects of several inhibitors on the cardiovascular actions of dopamine and tryptamine[2] in addition to those of norepinephrine and serotonin.

The inotropic (by the strain gauge arch technique[3]) and pressor effects of these amines were studied in 13 anesthetized, vagotomized dogs. The amines were administered at least twice in each experiment by rapid intravenous injection. After their cardiovascular actions were determined an MAO inhibitor was administered intravenously. One hour after the inhibitor was given the amines were readministered as before. The 1-hour waiting period was necessary to prevent interference of amine effects by direct cardiovascular actions of the inhibitors. *In vitro* studies of liver biopsies taken from several of the experimental animals indicated that the doses of inhibitors used were adequate to produce almost total inhibition of MAO.

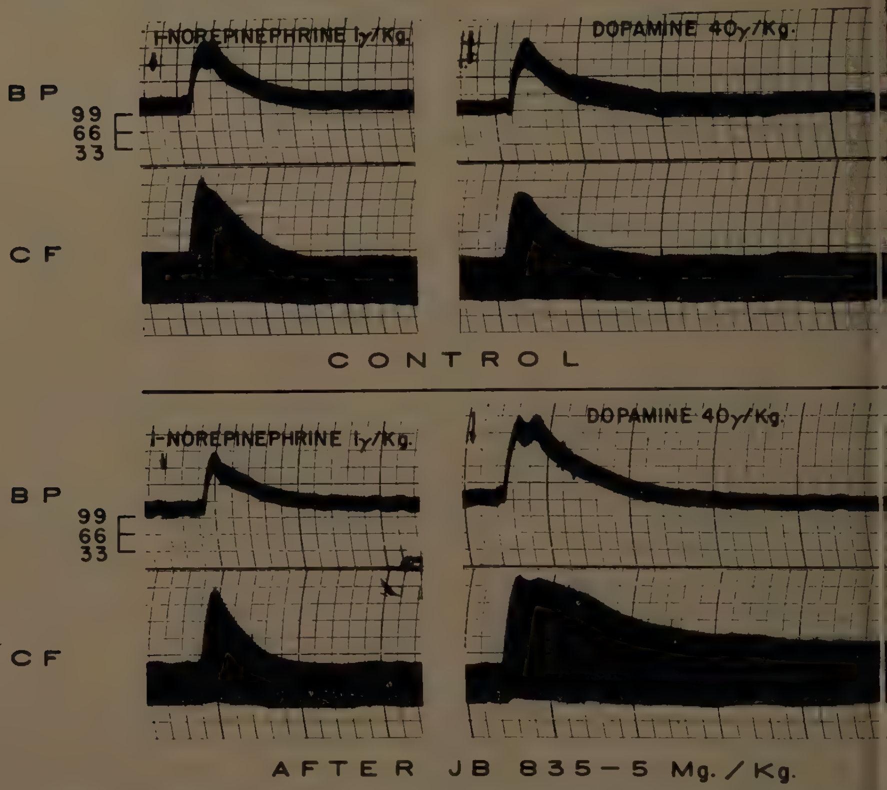


FIGURE 1. Effects of JB-835 on the cardiovascular actions of norepinephrine and dopamine. Upper tracings (control) illustrate the increments in blood pressure (BP) and contractile force (CF) of intravenous injections of 1 μg./kg. of l-norepinephrine and 40 μg./kg. of dopamine before administration of JB-835. The lower tracings illustrate the effects of the same amines about one hour after administration of JB-835 (5 mg./kg.). The scale to the left of the BP tracing is in mm. Hg and corresponds to the horizontal lines. The vertical lines signify 20-sec. time intervals.

Five inhibitors were used: the hydrazines JB-516 (1-phenyl-2-hydrazinopropane), JB-835 (4-phenyl-2-butyl-hydrazine), RO 5-0700 (2-benzyl-1-picolinyl-hydrazine) and the *harmala* alkaloids harmine and harmaline. Each was administered in a dose of 5 mg./kg. except for RO 5-0700, which was given in a dose of 10 mg./kg. JB-516 was found to exert a marked positive inotropic and pressor effect similar to the actions of amphetamine, which lasted about one hour. JB-835 had similar but less pronounced effects. RO 5-0700 exerted little effect on either the blood pressure or contractile force. The *harmala* alkaloids produced a pronounced but transient bradycardia and a moderate positive inotropic effect. Despite these diverse pharmacological effects, the changes produced in the actions of the amines were similar with all inhibitors.

FIGURES 1 and 2 illustrate tracings from a typical experiment with JB-835. The upper recordings (control) demonstrate the increase in arterial pressure (BP) and contractile force (CF) produced by intravenous injections of 1 μg./kg.

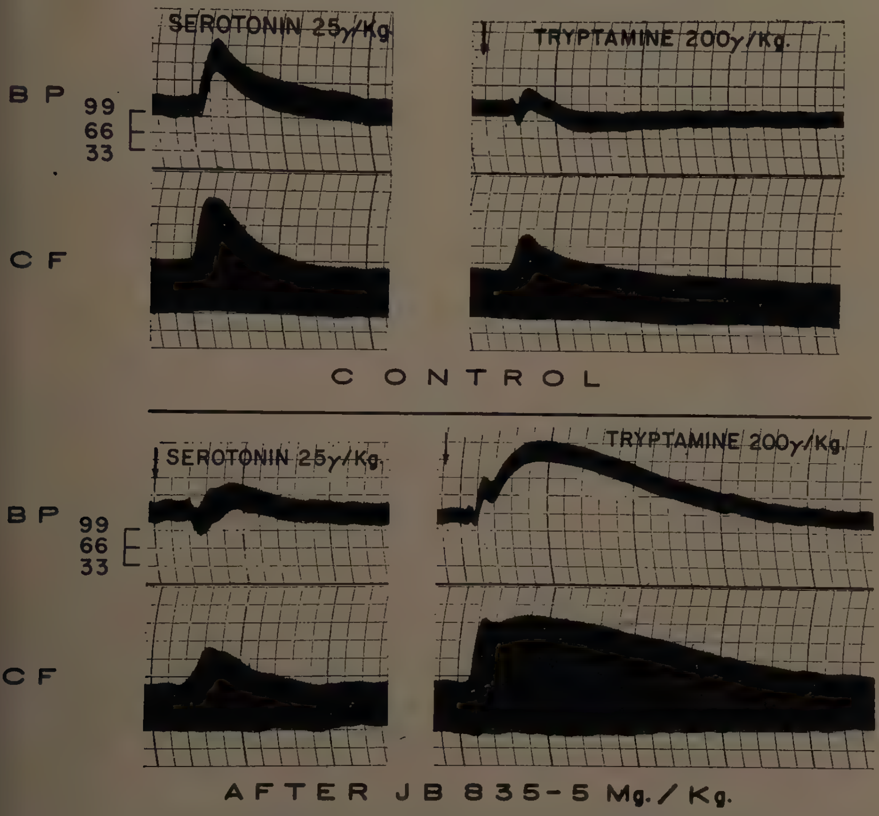


FIGURE 2. Effects of JB-835 on the cardiovascular actions of serotonin and tryptamine; same animal and conditions as in FIGURE 1.

of l-norepinephrine and 40 μg/kg. of dopamine in an anesthetized dog before the administration of JB-835. As may be noted, the injection of l-norepinephrine resulted in an increase in CF of about 150 per cent above control values and an increase in BP of about 100 mm. Hg. The injection of dopamine produced a CF increase of about 125 per cent above control values and a BP rise of about 100 mm. Hg. Both amines had similar brief periods of action in that 50 per cent of the duration of the inotropic effect (D_{50}) was about 50 sec.

The lower set of tracings illustrates the effects of these amines in the same animal about one hour after intravenous administration of 5 mg./kg. of JB-835. As may be noted, the effects of l-norepinephrine were slightly reduced, but the actions of dopamine were potentiated and markedly prolonged. The CF increase was about 190 per cent above control values, or more than 40 per cent greater than before the inhibitor. The duration of the effect was markedly prolonged with the D_{50} now over 200 seconds, or 4 times longer than before the inhibitor.

FIGURE 2 illustrates similar tracings with serotonin and tryptamine in the same animal before and after JB-835. As may be noted, the actions of serotonin were decreased, but the effects of tryptamine were markedly potentiated after administration of the inhibitor.

The average increases in heart contractile force produced by dopamine and tryptamine after administration of the inhibitors in the 13 experiments were 68 per cent and 50 per cent greater, respectively, than before the inhibitor. The hypertensive effects of these amines were also increased after administration of the inhibitors in most experiments, but biphasic effects made quantitation difficult. The inotropic and pressor effects of norepinephrine were not significantly altered. The average of the inotropic actions of serotonin also was not significantly affected, but in almost every experiment the pressor effect of this amine was significantly reduced.

A smaller number of experiments with other amines has indicated that the inotropic effects of tyramine,[1] another biogenic amine, were potentiated and prolonged by the inhibitors, whereas the effects of epinephrine, phenylephrine, and isoproterenol were not affected. With the collaboration of D. Horwitz and A. Sjoerdsma, preliminary studies in the human have demonstrated marked prolongation and potentiation of the hypertensive effects of dopamine after chronic, oral administration of JB-516.

These studies have demonstrated that the cardiovascular actions of dopamine and tryptamine are potentiated and prolonged in the intact animal, and that the effects of norepinephrine and serotonin are either not affected or diminished by prior administration of MAO inhibitors. The potentiation of dopamine and tryptamine appears to be correlated with MAO inhibition, but it is not known whether such potentiation is a simple enzymatic or more complex action of the inhibitors. These results indicate that dopamine, tryptamine, tyramine, and other as yet undiscovered potentiated amines should be considered along with serotonin and norepinephrine in evaluating the pharmacological effects of MAO inhibitors.

The action of the inhibitors to block the pressor effect of serotonin is being further investigated.

References

1. WEISSBACH, H., W. KING, A. SJOERDSMA & S. UDENFRIEND. J. Biol. Chem. In press.
2. SJOERDSMA, A., W. LOVENBERG & S. UDENFRIEND. Unpublished results.
3. BONIFACE, K. J., O. J. BRODIE & R. P. WALTON. 1953. Proc. Soc. Exptl. Biol. Med. **84:** 263.

ANTICONVULSANT PROPERTIES OF MONOAMINE OXIDASE INHIBITORS

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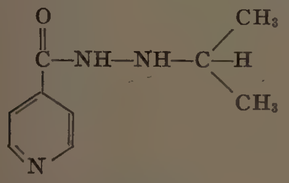
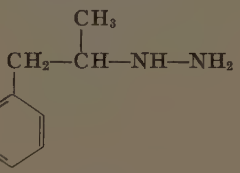
In contrast to most sedative drugs, reserpine lowers the threshold for electroshock- or pentylenetetrazol-induced convulsions in mice.[1, 2] Since monoamine oxidase (MAO) inhibitors elevate the levels of brain serotonin (5-HT) and norepinephrine (NE), whereas reserpine lowers the levels, it occurred to us that MAO inhibitors might act as anticonvulsants.

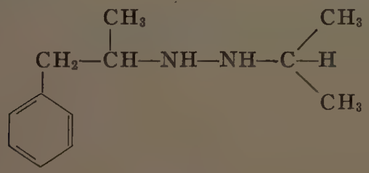
To explore this possibility, several MAO inhibitors were tested for their influence on convulsions produced in rats by supramaximal electroshock.[3] Three irreversibly acting inhibitors were studied: iproniazid, JB-516, and JB-807.



Iproniazid



JB-516



JB-807

Each of these drugs prevented tonic extension of the hind limbs, a criterion widely used in screening for antiepileptic drugs (TABLE 1). JB-516, which blocked tonic extension in a dose of 10 mg./kg., demonstrated a good "therapeutic ratio"; it evoked no obvious signs of toxicity in doses of 40 mg./kg., and the LD_{50} was approximately 100 mg./kg. By comparison, the LD_{50} for JB-807 was 80 mg./kg., and for iproniazid, 180 mg./kg. (free base).

Harmaline, a quick-acting reversible MAO inhibitor,[4] also prevented tonic extension when given in doses of 60 mg./kg.

Isoniazid (isonicotinylhydrazide), a congener of iproniazid, does not inhibit MAO and had no anticonvulsant effect. In fact, large doses enhanced electrically induced seizures, possibly because of an antipyridoxal action.[5]

The anticonvulsant actions of JB-516 and JB-807 were also tested in mice against intravenous injections of pentylenetetrazol. Both drugs blocked the tonic extensor phase and decreased mortality (TABLE 2). JB-516 was more toxic to mice than to rats, apparently because of a marked amphetaminelike action that is unrelated to the inhibition of MAO.[7] The mice were extremely excited for the first hour, and many of them died during this period even after doses as low as 40 mg./kg. However, if animals survived the first hour, they displayed no further adverse effects. JB-807, on the other hand, showed no amphetaminelike action.

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TABLE 1
EFFECT OF VARIOUS DRUGS ON ELECTROSHOCK SEIZURES

	No. of rats with full seizure pattern	Mean latency for tonic extension
	per group of 10	seconds
Controls	6.7 ± 1.23 S.D.* (15 groups)	4.0 ± 1.19 S.D.
Iproniazid 100 mg./kg., 15 hrs.	1/10, 0/10, 0/10	—
JB-516 10 mg./kg., 6 hrs.	0/10, 0/10, 0/10	—
JB-807 50 mg./kg., 6 hrs.	0/10, 0/10, 0/10	—
Harmaline 60 mg./kg., 1 hr.	1/10, 0/10	—
Diphenylhydantoin 25 mg./kg., 1 hr.	0/10, 0/10, 0/10	—
Reserpine 5 mg./kg., 4 hrs.	10/10, 10/10, 10/10	1.1 ± 0.05 S.D.
Isoniazid 50 mg./kg., in 2 equal doses at 2 and 15 hrs.	5/10, 7/10	4.3 ± 1.32 S.D.
Isoniazid 100 mg./kg., in 2 equal doses at 2 and 15 hrs.	10/10, 10/10, 7/10	2.5 ± 1.06 S.D.

* Standard deviation.

TABLE 2
PENTYLENETETRAZOL CONVULSIONS IN MICE

	Dose	Toxicity	No. with tonic extension	Ml. pentylenetetrazol for death	No. of survivals
Control			74/77	.45 ± .10 S.D. (0.25–0.65)	0/10
Phenobarb.	50 mg./kg.	Marked sedation	0/10	1.00	10/10
JB-807	100 mg./kg.	None	2/10	0.73	2/10
	150 mg./kg.	±	0/10	0.73	3/10
JB-516	40 mg./kg.	±	1/10	0.72	0/10
	30 mg./kg. 30 mg./kg.	LD_{10}	0/10	0.96	9/10

Effect of JB-807, JB-516, and phenobarbital on convulsions induced with timed intravenous infusion of 0.5 per cent pentylenetetrazol.[6] The mice were given the convulsant 2 hours after intraperitoneal administration of phenobarbital or 4 hours after intraperitoneal administration of JB-807 or JB-516. The second group of animals receiving JB-516 was given 60 mg./kg. in 2 divided doses 8 and 4 hours prior to testing.

JB-807 did not change the seizure pattern or affect mortality after intravenous caffeine or strychnine (TABLE 3 and 4). Its failure to afford protection against strychnine convulsions emphasizes the central origin of the anticonvulsant effect of MAO inhibitors.

Although MAO inhibitors have a definite anticonvulsant effect in experimental seizures, it is difficult to predict how useful the drugs might be in treating epilepsy. The clinical value of drugs used for the control of epilepsy may not correlate with results in animals.[8] This may be particularly true for MAO inhibitors, since preliminary studies *in vivo* indicate that mice and rats are much more resistant to the enzyme effects of MAO inhibitors than are rabbits, dogs,[7] and man.[9] Although MAO inhibitors are comparable in activity to diphenylhydantoin in blocking tonic extension (TABLE 1), they are not as effective in blocking tonic flexion, the first phase of a complete seizure pattern. However, MAO inhibitors affect pentylenetetrazol convulsions (TABLE 2), whereas diphenylhydantoin in single doses does not.[10]

TABLE 3
CAFFEINE CONVULSIONS IN MICE

	Dose	Time	No. with tonic extension	Ml. for tonic extension	Average survival
Control			10/10	0.52 (0.45 to 0.65)	2 min. (0 to 100)
JB-807	100 mg./kg.	4 hr.	6/6	0.53 (0.45 to 0.65)	4 min. (0 to 100)

Effect of JB-807 on convulsions induced with timed intravenous infusion of 1 per cent caffeine.[6] Mice were given caffeine 4 hours after intraperitoneal administration of JB-807.

TABLE 4
STRYCHNINE CONVULSIONS IN MICE

	Dose	No. with tonic extension	Cc. strychnine for death	Average survival (min.)
Control		10/10	0.40 (0.35 to 0.50)	0
Phenobarb.	50 mg./kg.	10/10	0.54 (0.45 to 0.65)	100
JB-807	100 mg./kg.	10/10	0.38 (0.30 to 0.45)	0

Effect of JB-807 on convulsions induced with timed intravenous infusion of 0.005 per cent strychnine.[6] Mice were given strychnine 2 hours after intraperitoneal administration of phenobarbital and 4 hours after administration of JB-807.

Possible Mechanism of the Anticonvulsant Effects of MAO inhibitors

Aside from any therapeutic implications, the anticonvulsant properties of MAO inhibitors offer an unusual opportunity to study some of the actions of MAO and its substrates in brain.

Implications of time-response curves. Chen *et al.*[1] reported that reserpine facilitated experimental convulsions after a latent period of about 2 hours, and that the effect persisted for several days. The effect of reserpine on brain 5-HT and NE is similar in its onset and duration of action; the decline in amine levels occurs over a period of about 2 hours and persists for several days.[11] This suggests that the brain's response to the experimental convulsions may depend on its content of 5-HT and NE.

Further support for this possibility is seen in the relationship between the level of amines and the anticonvulsant effect after giving MAO inhibitors to rats. Iproniazid elicited no anticonvulsant effect until the amine levels showed a definite rise, and the drug achieved its maximal effects when the amine levels reached their peaks (FIGURE 1). JB-516 acted more quickly than did iproniazid on amine levels and in producing an anticonvulsant effect (FIGURE 2). Both drugs altered the electroshock response and the brain amines for 2 to 3 days. Similar results were obtained with JB-807, which had its maximal effects in 6 hours.

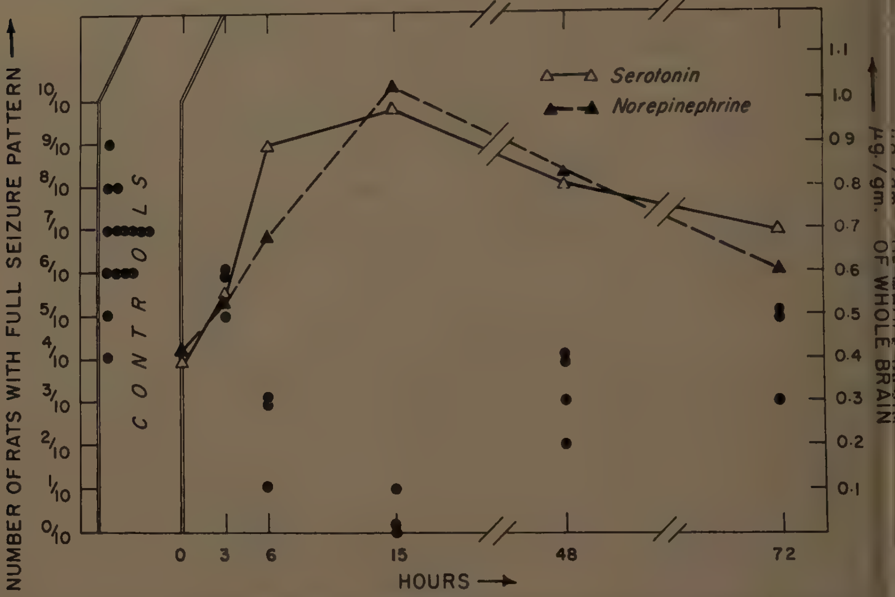

FIGURE 1. Effect of iproniazid (100 mg./kg.) on brain levels of serotonin and norepinephrine and on electroshock seizures in rats. The drug was injected intraperitoneally into female Sprague-Dawley rats weighing 125 to 150 gm. At various times thereafter, a 60-cycle AC current of 300 mAmp. was applied through corneal electrodes for 0.3 sec. (2 to 3 times maximal shock). Each solid circle represents the number of animals per group of 10 showing tonic extension of the hind limbs (full-seizure pattern). No animal was shocked more than once. Amine levels in whole brain were measured by previously described methods.[12, 13] Each triangle represents the average value of 6 animals.

Diphenylhydantoin (Dilantin*) in doses of 100 mg./kg. twice daily for 4 days has been reported to elevate brain serotonin levels in rats.[14] We observed that doses of 25 to 75 mg./kg. blocked the tonic extension component of electroshock, but did not increase the brain amines. The anticonvulsant action of diphenylhydantoin in rats does not appear, therefore, to be associated with a measurable rise in brain amine levels.

Antagonism to reserpine. Studies in which both an MAO inhibitor and reserpine were given to rats indicate that these compounds do not affect electroshock seizures directly, but act through changes in brain amines. When animals were treated with reserpine 2 hours before iproniazid, the levels of amines declined sharply, and only the enhancement of electroshock seizures produced by reserpine alone was observed (FIGURE 3). In contrast, when rats were treated with reserpine 2 hours after iproniazid, 5-HT and NE were released but not metabolized,[15] and a pronounced anticonvulsant effect was observed. The anticonvulsant effect was evident even though the amine levels were about the same as normal. These results strongly suggest that it may be the brain content of free amines rather than the total content of amines that is important in affecting experimental seizures. The results do not indicate whether 5-HT or NE is the important amine, but the data strongly suggest that a substance released by reserpine and metabolized by MAO is involved.

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* Parke, Davis.

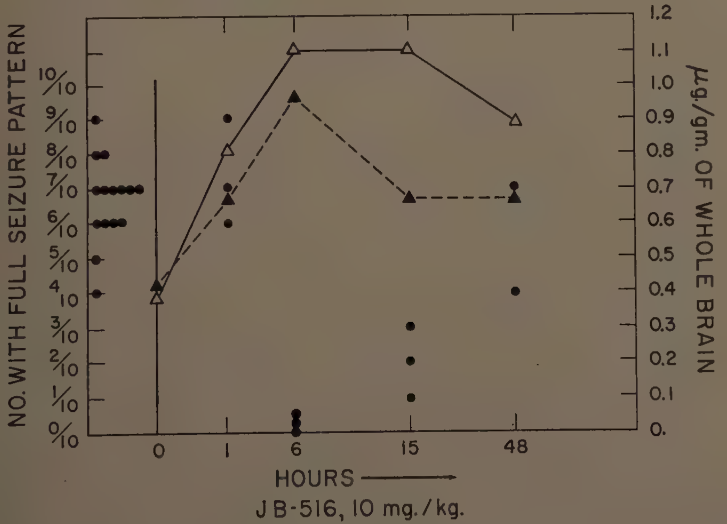

FIGURE 2. Effect of JB-516 (10 mg./kg.) on brain levels of serotonin and norepinephrine and on electroshock seizures in rats. Symbols as in FIGURE 1.

Effect of 5-HT and NE precursors. An attempt was made to ascertain which amine, 5-HT or NE, was more important in affecting electroshock convulsions by observing the effects of the amino acid precursors 5-hydroxytryptophan (5-HTP) and 3,4-dihydroxyphenylalanine (DOPA). Although a dose of 100 mg./kg. 5-HTP increased brain 5-HT 8 to 9 times, the response to electroshock was normal (FIGURE 4); some anticonvulsant effect was seen with a dose of 500 mg./kg. DOPA had no effect even in doses of 1000 mg./kg. Furthermore, when animals were treated with reserpine (5 mg./kg.) and then given both 5-HTP (250 mg./kg.) and DOPA (500 mg./kg.), their response to electroshock was indistinguishable from that of reserpinized controls, despite the fact that the animals were now alert and active.

The failure of 5-HTP and DOPA to influence the response to electroshock may be explained on the basis that elevation of amines produced by exogenous precursors is not pharmacologically equivalent to the changes produced by

MAO inhibitors. For example, DOPA yields Dopamine,[16] and it is not known how much, if any, NE is formed.

It is of interest that when 5-HTP was given to animals treated 2 hours previously with iproniazid, a distinct anticonvulsant effect was obtained with as little as 25 to 50 mg./kg. These results raise the possibility that pretreatment with iproniazid allowed 5-HT to accumulate in physiologically important sites. When 5-HTP is given alone, the activity of MAO may prevent the accumulation of 5-HT in these sites, even though the total brain content is greatly elevated. Pretreatment with iproniazid did not result in an anticonvulsant effect with DOPA.

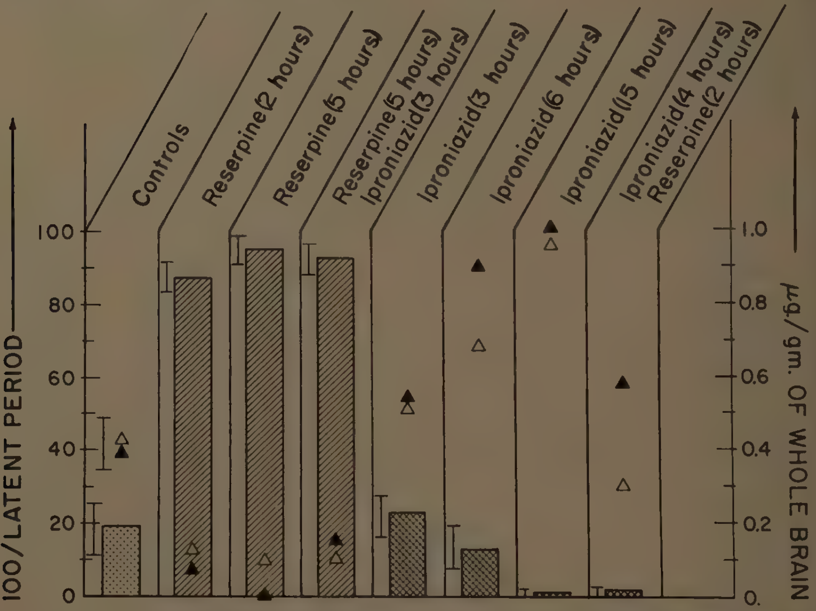

FIGURE 3. Effect of iproniazid (100 mg./kg.) and reserpine (5 mg./kg.) on brain levels of serotonin and norepinephrine and on electroshock seizures in rats. Response to electroshock here is described by the arbitrary unit of 100/latent period (in seconds) from the time when the current was applied to the appearance of tonic extension. Animals that did not develop tonic extension were considered to have an infinite latent period; therefore, the value of the unit for them was 100/∞, or zero. The values were averaged per group of 10 animals. Each bar represents at least 3 groups of 10 animals. The total range of the values is indicated to the left of the bars. The symbols for serotonin and norepinephrine are the same as in the previous figures.

In summary, MAO inhibitors in animals have a pronounced anticonvulsant effect that may warrant clinical trial. These effects are closely associated with an elevation of brain 5-HT and NE. Since reserpine releases brain amines and enhances convulsions, while MAO inhibitors increase the amines and protect against seizures, it is probable that certain physiologically active amines are important factors in determining the sensitivity of brain to experimental seizures.

The findings do not prove that the observed phenomena are due to NE or to 5-HT; they may be due to unknown substances that are also metabolized by MAO and released by reserpine.

The association between changes in physiologically active brain amines and enhancement of or protection against experimental seizures raises a possibility that certain types of epilepsy may involve a localized dysfunction in the formation, release, or metabolism of known or unknown amines.

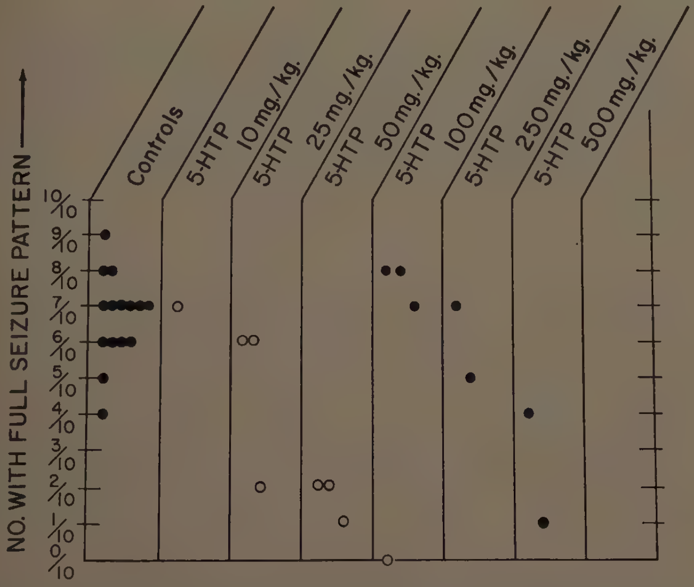

FIGURE 4. Effect of 5-HTP alone and 5-HTP after pretreatment with iproniazid on electroshock seizures in rats. Solid circles represent controls, or animals given 5-HTP intraperitoneally and tested 1 hour later. Open circles represent animals pretreated with iproniazid (100 mg./kg.) for 2 hours before 5-HTP was administered and then tested 1 hour after receiving the 5-HTP. All drugs were administered intraperitoneally as isotonic solutions or suspensions in volumes 1.5 cc. or less. Approximately one half of the controls received 1.5 c.c. of saline 1 to 3 hours before being tested with electroshock.

References

1. Chen, G., C. R. Ensor & B. Bohner. 1954. A facilitation action of reserpine on the central nervous system. Proc. Soc. Exptl. Biol. Med. **86**: 507.
2. Jenney, E. H. 1954. Changes in convulsant threshold after *Rauwolfia serpentina*, reserpine and veriloid. Federation Proc. **13**: 370.
3. Toman, J. F. P., E. A. Swinyard & L. S. Goodman. 1946. Properties of maximal seizures, and their alteration by anticonvulsant drugs and other agents. J. Neurophysiol. **9**: 231.
4. Udenfriend, S., B. Witkop, B. G. Redfield & H. Weissbach. Biochem. Pharmacol. In press.
5. Reilly, R. H., K. F. Killam, E. H. Jenny, W. H. Marshall, T. Tausig & N. S. Apter. 1953. Convulsant effects of isoniazid. J. Am. Med. **152**: 1317.
6. Orloff, M. J., H. L. Williams & C. C. Pfeiffer. 1949. Timed intravenous infusion of metrazol and strychnine for testing anticonvulsant drugs. Proc. Soc. Exptl. Biol. Med. **70**: 254.

7. Brodie, B. B., S. Spector & P. A. Shore. 1959. Interaction of monoamine oxidase inhibitors with physiologic and biochemical mechanisms in brain. Ann. N. Y. Acad. Sci. This monograph.
8. Swinyard, E. A., W. C. Brown & L. S. Goodman. 1952. Comparative assays of antiepileptic drugs in mice and rats. J. Pharmacol. Exptl. Therap. **106:** 319.
9. Sjoerdsma, A., L. Gillespie & S. Udenfriend. 1958. A simple method for the measurement of monoamine oxidase inhibition in man. Lancet. **2**(7038): 159.
10. Goodman, L. & B. Lih. 1941. Effects of Dilantin on metrazol convulsions. J. Pharmacol. Exptl. Therap. **72:** 18.
11. Brodie, B. B., J. S. Olin, K. G. Kuntzman & P. A. Shore. 1957. Possible interrelationship between release of brain norepinephrine and serotonin by reserpine. Science. **125**(3261): 1293.
12. Bogdanski, D. F., A. Pletscher, B. B. Brodie & S. Udenfriend. 1956. Identification and assay of serotonin in brain. J. Pharmacol. Exptl. Therap. **117:** 82.
13. Shore, P. A. & J. S. Olin. 1958. Identification and chemical assay of norepinephrine in brain and other tissues. J. Pharmacol. Exptl. Therap. **122:** 295.
14. Bonnycastle, D. D., N. J. Giarman & M. K. Paasonen. 1957. Anticonvulsant compounds and 5-hydroxytryptamine in rat brain. Brit. J. Pharmacol. **12**(2): 228.
15. Shore, P. A. and Brodie, B. B. 1957. LSD-like effects elicited by reserpine in rabbits pretreated with iproniazid. Proc. Soc. Exptl. Biol. Med. **94:** 433.
16. Carlsson, A., M. Lindqvist, T. Magnusson, B. Waldeck. 1958. On the presence of 3-hydroxytyramine in brain. Science. **127**(3296): 471.

Discussion of the Paper

J. P. Buckley (*University of Pittsburgh School of Pharmacy, Pittsburgh, Pa.*): Prockop and his colleagues have described another action of monoamine oxidase inhibitors that can be added to the already great variety of known activities. I was extremely interested in the fact that, although 5-hydroxytryptophan greatly increased brain serotonin levels, it did not alter the response to electroshock. It does appear that all of the activities of the monoamine oxidase inhibitors cannot be attributed to increased serotonin levels, and that some may be due to the effects of the compounds themselves on receptor sites.

Here I shall report the results of several studies carried out in collaboration with A. Furgiuele and W. J. Kinnard at the University of Pittsburgh. We have observed that β-phenylisopropylhydrazine (JB-516), at a dosage of 15 mg./kg. intraperitoneally (I.P.) every fourth day, produced ataxia and symptoms resembling skeletal muscle relaxation in the rat after 6 to 8 doses. This same dosage schedule administered subcutaneously to dogs produced similar symptoms in approximately the same period of time. Mice receiving JB-516, 2 to 50 mg./kg., demonstrated a decreased ability to perform forced motor activities as measured with the Rotarod apparatus described by Dunham and Miya[1] and by Kinnard and Carr.[2]

Since JB-516 appeared to produce some degree of skeletal muscle relaxation and has been reported to possess anticonvulsant properties, we thought it might antagonize the actions of tremorine (1,4-dipyrrolidino-2-butyne), a compound that produces characteristic tremors of the head and hind limbs, as well as marked parasympathetic effects. Tremorine (20 mg./kg.) was administered to male Wistar rats as follows:

Group 1. Eighteen rats weighing between 100 and 125 gm.

Group 2. Twelve rats (100 to 125 gm.) pretreated with atropine sulfate (4 mg./kg.) 45 min. prior to receiving the test dose of tremorine.

Group 3. Twelve rats (100 to 125 gm.) pretreated with JB-516 (15 mg./kg. I.P.) 6½ hours prior to receiving the test dose of tremorine.

Group 4. Nine rats (100–125 gm.) pretreated with 2 doses of JB-516 of 15 mg./kg. each. The initial dose was administered 26 hours prior to the test dose of tremorine and the second dose 20 hours later.

Group 5. Nine rats (200 to 225 gm.) pretreated with 16 doses of JB-516 (15 mg./kg.) every fourth day. All animals demonstrated severe ataxia and symptoms resembling skeletal muscle depression.

JB-516 did not prevent the tremors or parasympathetic effects of tremorine in the rat. Even the animals pretreated with 16 doses of JB-516 developed severe tremors and other such effects. Atropine sulfate blocked both the tremors and parasympathetic activities of tremorine.

We were interested in determining the mechanism of the skeletal muscle incoordination and weakness produced by JB-516. The effect of β-phenylisopropylhydrazine on the ipsilateral flexor reflex of cats and dogs was studied. Cats received repeated doses of JB-516 (7.5 or 10 mg./kg.) at intervals of 15 or 30 min. Dogs received 10 mg./kg. of JB-516 every 20 min. JB-516 did not affect the flexor reflex in the cat. The cats died between the twelfth and nineteenth doses. The flexor reflex was abolished in dogs between the third and sixteenth doses. Dogs receiving normal saline solution (2 ml./20 min) maintained this reflex after 21 doses (7 hours). Because of the long duration of the experiments involved it is difficult to make any definite conclusions at this time, as there is always the possibility of neuronal degeneration. However, JB-516 does appear to possess some degree of interneuronal blocking activity in the dog. It is of interest to note that JB-516 does not produce ataxia and muscle weakness in the cat unless the dosage is manipulated very carefully, and this may explain the inability of this compound to alter the flexor reflex in this species.

References

1. Dunham, N. W. & T. S. Miya. 1957. A note on a simple apparatus for detecting neurological deficit in rats and mice. J. Am. Pharmacol. Assoc. Sci. Ed. **46:** 208.
2. Kinnard, W. J., Jr. & C. J. Carr. 1957. A preliminary procedure for the evaluation of central nervous system depressants. J. Pharmacol. Exptl. Therap. **121:** 354.

COMPARISON OF IPRONIAZID WITH OTHER AMINE OXIDASE INHIBITORS, INCLUDING W-1544, JB-516, RO 4-1018, and RO 5-0700

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In Ho Park, Dina Rochlin, John C. Saunders, Maurice Vaisberg
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In April 1957, when we publicly introduced iproniazid for the treatment of certain types of apathy and depression, we postulated that the action of the drug was probably due to its function as an amine oxidase inhibitor.[1] Since that time we have evaluated a half-dozen other amine oxidase inhibitors and are naturally pleased to be able to report that every one of them, to a greater or lesser degree, acts in the same direction as does iproniazid. We have also worked with a number of other antidepressive compounds that are not amine oxidase inhibitors. Although some of these had favorable psychostimulating characteristics, there is an appreciable difference in the effects that they exert; although there is some overlapping, tentative clinical differences can be described between patients who will respond to the amine oxidase inhibitors in contrast to those who respond to other types of compounds. In addition to constituting a tremendous therapeutic advance, as evidenced by the fact that over a half-million patients have been treated with these compounds since their introduction only one and one-half years ago, we believe that the breakthrough in respect to understanding the biochemistry of mental illness is of even greater significance. Although the *Rauwolfia* alkaloids and the phenothiazine derivatives are our major instruments of drug therapy for certain other psychiatric syndromes, very little is understood of their mode of action despite a number of years of intense investigation. For the first time we have been able to describe a principle of pharmacological activity rather than merely to identify a chemical structure. Knowledge of how such a drug is working points the way to investigation of those factors that may have been productive of the condition in the first place.

Subjects

The groups of patients on which our report is based are as follows.

(1) Fifty nonhospitalized cases of either depression or other psychiatric disorders in which depression is a prominent symptom. In most cases each patient received in succession at least 2 of the psychic energizers and at times 3 of them. In addition, some were also placed on psychoactivating drugs other than amine oxidase inhibitors. This group was primarily female, although a number of males was included. In view of the exigencies of private practice, it was not possible to utilize the same type of experimental design as was carried out on the other patient groups, so that illustrative cases are selected to make clear certain points rather than merely to tabulate clinical findings.

(2) Previously reported groups of patients treated by us with iproniazid (Marsilid*) are used as a reference point for other drugs. Other base-line data

* Hoffmann-La Roche.

are drawn from the more than 500 patients at Rockland State Hospital who have been treated with iproniazid. These include a large variety of diagnoses, but are primarily those cases characterized by depression, apathy, and withdrawal.

(3) Phenelzine* (W-1544) was evaluated on: (a) 24 adult male chronic schizophrenics characterized by depression, apathy, and/or withdrawal; (b) 5 semichronic or acute patients of the same type; (c) 20 catatonic or markedly depressed males; and (d) ambulatory patients seen in private practice.

(4) Catron† (JB-516) was used on 5 separate categories of patients: (a) a group of 5 adolescents characterized by apathy, withdrawal, or depression (in this group patients were selected primarily because of lack of interest or inertia); (b) 4 recent female admissions selected on the basis of evidence of depression, a few cases belonging in the withdrawn and apathetic group; (c) 22 acute schizophrenics who had responded to chlorpromazine with loss of secondary symptoms, but who were still hospitalized because of such symptoms as apathy and flatness of affect; (d) 13 chronic female schizophrenics characterized by apathy, withdrawal, and/or depression; and (e) ambulatory patients seen in private practice.

(5) RO 4-1018 was initially evaluated on: (a) 16 depressed patients in the male reception service; (b) 5 acutely suicidal male admissions; (c) 10 chronic female schizophrenics with marked depression, apathy, and/or withdrawal; and (d) ambulatory patients seen in private practice.

(6) RO 5-0700 was given to: (a) 8 chronic female patients characterized by apathy, withdrawal, and depression; (b) 8 semichronic or acute female patients of the same type; (c) 10 chronic female psychotics of the apathetic type; and (d) ambulatory patients seen in private practice.

(7) Reference is made to G-22355, otherwise known as imipramine or Tofranil.‡ This is not a primary report on this drug, but some comparisons are nevertheless in order. We have previously discussed this preparation in a preliminary report.

The dose of each drug was within a specific range, with individualization within this range as treatment progressed (it should be emphatically stressed that much closer regulation of dosage is needed than with reserpine or the phenothiazine derivatives). Quite different effects might have been obtained at different levels but, for the initial investigation, we arbitrarily selected what appeared to be the most probable useful range. In the past we have found that a drug not very effective at one level gave excellent results at a higher one; in view of our clinical findings we are tentatively exploring other dosage ranges and regimens of administration. For instance, since it appears that a certain tissue level of psychic energizers is necessary for therapeutic effects, specified patients are receiving large initial doses to determine the possibility of more rapid response.

The recommendation of the manufacturers was that JB-516 be given for 4 days and discontinued for a like period, with repetition as often as necessary.

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* Warner Chilcott. Fifteen mg. of the commercial preparation (Nardil) is equivalent to 25 mg. of the preparation that we investigated.

† Lakeside.

‡ Geigy.

In our initial investigations we did not follow this technique and, as will be pointed out, we have some evidence to indicate that for the majority of patients it is not the most satisfactory procedure.

As we have repeatedly stated since we introduced iproniazid for the treatment of depression, 150 mg., and not 50 mg., should be the average initial dose in both hospitalized and ambulatory patients. However, we are continuing to evaluate various dosages.

Another factor that may considerably alter the therapeutic efficacy of these drugs is their use in combination with other medications. Amphetamine, almost invariably given to the private patients, seemed to expedite the time of reaction and enhance the clinical effect, but it was considered undesirable for the hospitalized patients, since it would have provided a contaminant in the evaluation of the drug. In some cases, both ambulatory and institutionalized, psychic energizers were used with phenothiazine derivatives to combat or prevent anxiety, tension, and hyperactivity. This therapy may be desirable routinely for schizophrenics, agitated depressions, and certain other types of patients, one not entirely dissimilar from the treatment of gastric ulcers by combining an antacid with an anticholinergic or even an antispasmodic. As would follow from the animal investigations, reserpine also has been used selectively with the psychic energizers. Each medication may play an essential but not complete role, and we are continuing to explore this area. Therefore, while this report is primarily an evaluation of the drugs used by themselves, we are alert to the fact that in some instances their combination with other effective agents in the field may achieve maximal benefits.

One final caution: no effort was made to equate the groups as to severity of illness, diagnostic category, duration of hospitalization, or sex (which we know to be a factor in the use of phenothiazines). Most patients were selected on the basis of symptomatology, and only after establishing such factors as effective dosage range, necessary duration of treatment, and selection of most susceptible cases can an accurate comparison be made. Nevertheless, certain conclusions appear probable because of the clinical evaluation experience of the investigators. Despite the fetish for objective test measurements and cross-over design, the best measuring instrument is still the clinical judgment of a team of experienced personnel.

Results

(1) *Phenelzine* (*W-1544*). After iproniazid, the next amine oxidase inhibitor we received for clinical testing was the Warner-Chilcott preparation W-1544. Thirty patients were selected for the first subgroup, with one exception: schizophrenics characterized by depression, withdrawal, disinterest, apathy, or very similar dominant symptoms. The exception, a psychotic with encephalitis, will be discussed separately. Of the 29 schizophrenics, 24 were chronic by the criterion of continuing hospitalization for 3 years or more, the most recent admission being in 1952 and the earliest in 1934. Both diagnosis and duration of illness affect the outcome of treatment, and we have either stratified or controlled these factors. We did not stratify age, for we have no evidence that per se it is a relevant variable.

Previous treatment included electroshock therapy except for 5 patients, of whom 2 had received pentamethylenetetrazol (Metrazol*), one a series of ataractic drugs, and 2 no previous extensive treatment. One had been lobotomized. The patients were characterized as "idle, seclusive, unclean, untidy, confused, uninterested, emotionally inappropriate, poor contact, dull, depressed, and in some of the cases manneristic, disoriented, mute, hallucinated, etc."

The dosage was 10 mg. twice daily for 2 weeks, 20 mg. twice daily for 3 weeks and thereafter, in most cases, 20 mg. 3 times daily. In patients who failed to respond adequately, it was increased to 100 mg. daily for 1 month prior to discontinuation. Treatment was continued for a minimum of 6 months up to a maximum of 9 months in this subgroup of 24 and, since the 5 patients who were hospitalized less than 3 years could be regarded a semichronic, having having been admitted in either 1955 or 1956, the same symptoms and dosage routine were utilized. The patient with psychosis following encephalitis had been admitted in 1947.

Among the chronic schizophrenics, all showed some degree of improvement in respect to the symptoms for which W-1544 was administered. Activity was increased in every patient, 4 exhibiting such aggressiveness, hostility, agitation, and occasional assaultiveness that medication was necessarily reduced. Even on reduced dosage, hyperactivity continued in 3 of the 4 patients, and medication was eventually discontinued. In one patient who became euphoric, it was possible to reduce medication rather than discontinue it. None of the chronic patients improved sufficiently to be discharged from the hospital, but all were either schizophrenic or psychotic with encephalitis and, as originally pointed out with iproniazid, the drug is not intended as a specific for schizophrenia. Since at least one full year of medication had been given to all the chronic patients discharged from the original group treated with iproniazid, the two sets of results are roughly comparable as to duration of administration. Our impression, based on careful clinical observation, is that, at the dosages used, W-1544 is somewhat shorter-acting than iproniazid in its clinical effects. It may be more specifically indicated for use with ambulatory patients, where there is evidence that in some cases lower doses and shorter duration of treatment are effective. This could provide for some selectivity and choice, dependent on clinical needs, since there will be patients in whom shorter action is desirable. The drug did not appear to potentiate barbiturates in the same manner as iproniazid; if confirmed, this may make it particularly useful under certain circumstances.

About one half of the patients hospitalized less than 3 years have been able to go home on convalescent status. These were responding well after only a few months of treatment. In 2 other cases treatment was continued for 7 and 9 months, after which they were released from the hospital. The patient with psychosis following encephalitis improved, but not sufficiently to be discharged. Results in the more recently hospitalized patients indicate that a major use of the drug would be with ambulatory milder or less chronic depressions. We again emphasize, however, that in many chronic cases depression was alleviated, the patients remaining hospitalized, not because of withdrawal, apathy, or retardation, but because of their schizophrenic symptoms. Among the markedly deteriorated patients, improvement was less than would have been anticipated from the use of iproniazid. On the other hand, the catatonic patients responded better to W-1544 than to any of the other amine oxidase inhibitors.

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* Bilhuber-Knoll.

W-1544 constitutes a definite advance in the treatment of the conditions cited, appearing in certain instances to be better applicable than other amine oxidase inhibitors and making possible a greater selectivity in the use of these drugs. Illustrative of the type of response obtained with W-1544 is the following private case history, which also emphasizes the individual specificity of a particular drug.

An attractive 30-year-old housewife had suffered from marked depression and fatigue which she attempted to relieve by the use of sizable amounts of alcohol, barbiturates, and up to 30 to 40 mg. of dextro-amphetamine (Dexedrine*) a day. Three years of psychoanalytic treatment had not provided any measurable relief. She was given Warner-Chilcott compound W-1544 in doses of 2 10-mg. tablets 3 times daily, plus 5 mg. of Dexedrine. At the end of the first week of treatment her husband reported that for the first time since he had known her she was "not at one extreme or the other," and she herself noted she was much less irritable and was "enjoying myself quietly." By the end of 6 weeks she had improved to the extent that her "ego pills," as she called them, could be reduced to 10 mg. 3 times daily, but in another 2 weeks she began complaining somewhat of constipation, dizziness, and a slight return of her "morning blues." Instead of reducing the dosage of W-1544 as indicated, iproniazid was substituted in doses of 50 mg. 3 times daily; at the end of 10 days the patient reported none of the feeling of elation on iproniazid that had been achieved with W-1544. She commented in retrospect that the drugs were real life-savers, since when previously depressed she would get drunk and go driving and, on other occasions, she would sit for hours loaded with barbiturates and try to make up her mind to jump out of the window.

A series of tests, given as the result of an initial diagnosis of possible hypothyroidism, indicated a very slight reduction in thyroid function. The patient was placed on thyroid by her internist, which seemed to produce a fair amount of sluggishness. She also developed edema and was treated with 2-acetylamino 1,3,4-thiadiazole-5-sulfonamide (Diamox†). Iproniazid was reduced to 50 mg. per day and, 1 week later, the edema had subsided somewhat. The patient still did not feel her previous energy, so with no authorization she combined 40 mg. of W-1544 with iproniazid and, in her own words, "went like a house on fire." The edema continued, however, and she was then given JB-516 to determine if it would bring about relief. Two weeks later, for the first time in her life (despite 2 marriages) she had an orgasm. Her desire to sleep a large part of the day had completely disappeared, and she was taking only 2 to 3 hours' sleep without any fatigue, not wishing to spend her time in bed since "there were so many interesting things to do." An apparent exception to this was her extremely active sex life, the patient reporting that her husband was completely exhausted, but "we've never been so happy." However, at the end of another few weeks she increased somewhat in irritability, then became more sluggish as the dose of thyroid was increased. With the approval of the internist she was taken off thyroid and one week later she began feeling better but, because of residual irritability, prochlorperazine (Compazine*) in 10-mg. Spansules was added.

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* Smith, Kline & French.
† Lederle.

During the summer she was carried on the JB-516 by one of my colleagues. On my return in the fall she noted no recurrence of depression since the initial W-1544 but, as a result of the loss of anxiety and tension, she had become somewhat lethargic. All medication was discontinued; for the first week she felt well, but after 2 weeks her headaches and stomach pains, absent for more than 8 months, returned. By the third week she felt as ill as during pretreatment and, obviously, would not exist adequately without medication; since W-1544 had seemed to provide the best results, she was again placed on the original medication and responded within 10 days.

She herself drew a comparison between this compound, JB-516, and iproniazid. The last 2 made her industrious and ambitious to "work like hell," providing an inside energy but no great desire to socialize with other people; she kept her house in first-rate order, but was not too interested in getting out of it. With W-1544 she experienced a "festive type of energy," wanting to get out and be with other people, enjoying her social contacts greatly, and able to concentrate much better on her reading. Her only complaint was that the sexual frigidity, relieved by JB-516, had again returned, but subsequently this condition also was alleviated. She made special note of the fact that her daughter had been failing in school (with average grades of 30). After medication the woman had become a more cheerful, interested mother, and the child's grades had gone up so that, after a few months, she was averaging 90.

The technique of starting with small groups of patients and, on the basis of symptom response, selecting other apparently amenable cases is exemplified in our work with W-1544. Since the best responses were among those with depressions and since the catatonics did surprisingly well, 19 patients primarily characterized by these symptoms were selected for the next phase of the investigation. In this group, loaded with psychotic patients of acute illness having shorter duration of treatment, no discharges would have been anticipated as yet, so final results must be reported later. Because doses above 60 mg. a day produced hyperactivity in some patients, we have felt it best to explore the preparation thoroughly at this level before venturing into higher regions. All routine laboratory tests were within the limits of normal variations on all patients actually receiving the drug. This does not mean that there were no shifts in some of the findings within the normal range; with the Warner-Chilcott preparation these included a shift toward higher normal levels of blood urea nitrogen (BUN).

(2) *Catron* (*JB-516*). The next amine oxidase inhibitor investigated was JB-516. We selected 5 male adolescents aged 12 to 16, acute admissions who had been in the hospital from 3 months to approximately 1 year at the time medication was started. Two were diagnosed as cases of childhood schizo-

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* Smith, Kline & French.

phrenia, 1 a neurotic depression, and 2 manifested primary behavior disorders (conduct disturbance). The most notable symptoms were flattening of affect, depression, sometimes accompanied by hostility, and passive or negativistic behavior of the passive type. The patients received a single dose of 6.25 mg. for 6 months. Although some reported feeling better and kept out of trouble with other children, the over-all results were less impressive than with the adults. Two showed some degree of improvement, but less than we had hoped. In one of these there was a shift from withdrawal to "acting out," the boy commenting, "Now I have nothing against myself, why should I kill myself?" We hope to recommence therapy on these 2 patients and see whether treatment of longer duration will produce a more lasting and marked affect.

When we state that the results were not as great as expected, this by no means indicates that the preparation was without favorable action. Moreover, in view of the reactions of children to the amphetamines, it might well be that larger doses are indicated. It is also quite possible that the combination of JB-516 with other psychic activators will be necessary to maximize its action.

The next hospitalized group consisted of 13 chronically withdrawn, depressed, apathetic female schizophrenics, including one lobotomized patient, who had previously failed to respond to treatment with ataractics and electroshock therapy. Daily dosage ranged from 12.5 to 25 mg. Four of the patients showed very definite improvement, including remission of suicidal symptoms in one; however, since the schizophrenia persisted, obviously they were not suitable for discharge. In 4 other patients the depression was alleviated but was replaced by hyperactivity, assaultiveness, and agitation, so that clinically they became even more difficult to manage. In only 5 was there so little change that we could not be sure that the results were not due to factors other than the medication.

The results in private patients. Although not certain, some investigators felt that the hospitalized patients responded more rapidly to JB-516 than to other amine oxidase inhibitors, including iproniazid. In ambulatory nonhospitalized patients, quite frequently an initial favorable reaction was noted within 4 days, in contrast to the 2 weeks usually required with iproniazid. There was a slight tendency to show lowered hemoglobin, but laboratory tests were all within normal limits, with no systematic shift in blood urea nitrogen. We are still investigating, but thus far have obtained no evidence of color blindness. At this time we see no essential difference in therapeutic response between the 2 preparations, although we are considerably expanding our series to look for possible finer differences. In certain nonhospitalized patients (when both iproniazid and JB-516 are used with an amphetamine) the action of JB-516 is more rapid but, since some patients respond to one and not to the other, it is valuable to have more than one such amine oxidase inhibitor available. For reasons as yet unclear, there is a specificity of action—certainly clinically and, probably, metabolically—similar to that of the antihistaminics, for example.

In a comparative study on patients hospitalized for less than 1 year, there appeared to be no significant difference between iproniazid and JB-516 with respect to either effectiveness or side reactions. The following points are therefore equally applicable to both.

The 52 patients on iproniazid and the 26 on JB-516 were all acute female admissions selected because of depression. To date, 53 per cent of the former have gone home, and 46 per cent of the latter have either gone home or are in the process of release from the hospital. All 19 nonschizophrenics on iproniazid and the 4 on JB-516 showed significant relief of their depressions. Sixteen of the 30 schizophrenics on iproniazid and 4 of the 22 on JB-516 showed an over-all worsening of their clinical conditions. The difference is probably due to the continued use of ataractics with all patients on JB-516 but only some on iproniazid.

The schizophrenics in both groups had been treated with ataractic medications, either reserpine, phenothiazine derivatives, or a combination of the two. As described by one of us (E.G.), they had reached a "plateau": their secondary psychotic symptoms—hallucinations, delusions, and ideas of reference—had disappeared, but there still remained Bleuler's primary psychotic pattern, including such symptoms as flatness of affect, autism, and disassociation.[2] The psychic energizer was added in an effort to move them out of this unfertile state. Every schizophrenic treated on the female admission service (E.G.) showed a significant shift in the nature of her psychosis, for better or worse, discharge or deterioration, remission or regression (as indicated above, the over-all picture may worsen as a result of exacerbation of previously latent psychopathology or the acting out of otherwise dormant content). Nine of the 22 patients on JB-516 either have been or are being discharged. In a prior group of the same type, 75 per cent became worse on iproniazid alone, as contrasted with one third when this drug was combined with a phenothiazine derivative or reserpine. The figures are essentially the same, any difference probably being due to the use of ataractics with one group but not the other. In summary, therefore, when either is used alone about one fourth of the patients improve and three fourths become worse; when used with ataractics, more than one half improve and most of these do so to the extent of being eligible for discharge; one third will show some change but not sufficient to warrant a change of status; a few will become worse. Despite a careful review of the case histories, we know of no way of predicting in advance which of the schizophrenics will respond favorably and which will not; therefore, at the present time, use of the drugs is a calculated risk.

Of the 16 patients who worsened on iproniazid, 10 required electroconvulsive therapy (ECT) and 3 required insulin because of increased agitation, particularly manifested by suicidal strivings which, interestingly enough, were not depressive but of a compulsive, paranoid, or disassociated type. Of the 4 patients who became worse on JB-516, one required insulin. There is some evidence that the psychic energizers may cause a fairly permanent change in psychosis, which does not regress to its former state after withdrawal of the medication, but may require active treatment to maintain improvement. This is illustrated in the following case history of a private patient.

Case No. 20, Patient F.M. This college student was sent home by his university psychiatrist with a marked depression that interfered with his ability to assimilate class work and resulted in his spending most of the time in his room. Over the past 2 years he had received intermittent "free-association" therapy. He was started on 8 mg. of perphenazine and 10 mg. of iproniazid 3 times daily. At the end of 3 weeks he reported that "the stuff is working and I begin to feel I have the ability to choose what I do," and that people no longer "scare me to death." There was definitely less depression. A week later he commented that, instead of going to pieces when excited, a remarkable steadiness now replaced his previous fear. He was able to read longer and concentrate better. Dosage of iproniazid was reduced at this time, but subsequently it became necessary to increase it up to and above the initial dosage, going as high as 50 mg. three times daily. Side effects consisted primarily of inability to achieve an orgasm on masturbation, with occasional delayed micturition. He was able to do with 7 or 8 hours' sleep a day instead of the usual 10. Later, some constipation and anxiety developed and gradually increased. At this point, roughly 3 months after starting on iproniazid, JB-516 (12.5 mg. a day) was substituted, along with 10 mg. of Compazine twice daily. At the end of two weeks he felt "great," was sleeping only 5 to 6 hours a night and, for the first time in years, felt like working. He returned to his university with the approval of the school psychiatrist.

At summer school he did extremely well, getting all A's, but while under the care of a psychiatrist during the summer vacation he became hyperactive and extremely talkative. The psychiatrist's recommendation that dosage be reduced was disregarded by the patient, but one week before being seen again in the fall he reduced it to 6.25 mg. a day. He was still behaving in a highly agitated, overactive manner, and medication was discontinued. At the end of the month he again had to leave the university, expressing marked psychopathology and behaving in a most eccentric manner. Although perphenazine (Trilafon*) again was prescribed, he refused to take any medication, considering himself at this point to be an almost perfect individual. After 2 months he gave no real evidence of returning to either his former condition or one within normal limits.

An important additional point is that during the first month of treatment patients on psychic energizers almost without exception demand additional attention and reassurance, a result of awareness of rapid and marked changes in their psychological status. If the therapist is not aware of this he may discontinue treatment, feeling that the patient has worsened. In the group described above, on both iproniazid and JB-516, the first major shift in reaction occurred in an almost clocklike fashion between the twelfth and fifteenth days. As previously noted, all patients show a significant change, and whether they improve or not, they tend to become more outgoing and communicative. We would therefore strongly urge that, for schizophrenic patients with depression, a phenothiazine derivative should be given routinely with the psychic energizer. The probability of success is enhanced, and the likelihood of worsening is markedly reduced.

One private case history will illustrate how rapid and brief treatment can be. *Case No. 15, Patient G.H.* This 36-year-old business executive, referred by a local psychiatrist who had not been successful with iproniazid (50 mg. a day), had an aversion to the drug since it had not been helpful. At this time he had been in depression for about 3 months, following 8 years of recurrent manic states and episodes involving depressions lasting as long as 6 months. After the first of such episodes he had been in a private sanitorium with no shortening of the depression. He was started on JB-516 (12.5 mg.) plus 10 mg. of prochlorperazine (Compazine) twice daily. Within 4 days all evidence of the depression disappeared and, at the end of 2 weeks, instead of literally beating his head on the floor and contemplating the most painless means of suicide, he joyously referred to the fact that "life is a cinch." The patient, with a supply of JB-516, was returned to the referring physician and has been doing extremely well. Medication was discontinued at the end of the 2-week period and there has as yet (2 months later) been no need to use it again. He is greatly reassured, feeling that if depression recurs he has an effective treatment available.

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* Schering.

(3) *RO 4-1018*. Since there are now several hundred analogues of iproniazid, it is a serious problem to select those most likely to be useful for clinical testing. On the basis of pharmacological studies and animal experimentation, this Hoffmann-La Roche preparation was one of those selected and the next in chronological order to be tested. To test psychic energizers in acutely suicidal patients, we instituted treatment with iproniazid on 10 such males at the time of institutionalization. In expanding the investigation of RO 4-1018, we subsequently added 5 more and can therefore draw tentative comparisons. They were all placed on treatment within 1 week after admission. Assuming that therapeutic results are only as good as with electroshock therapy (we believe them to be better), one of the great advantages of psychic energizers is the shortening of the elaborate and expensive series of tests required before patients can be given ECT. There is also the difficulty of scheduling electroencephalograms, electrocardiograms, skull and spine plates, and consultations with various specialists if there has been any previous somatic illness. Finally, after weeks or even months, when the patient is found to be suitable, the family may refuse permission. Except for patients with specific liver damage, we have found no strong contraindications to the use of the drug and, if it is used circumspectly, liver damage is not an absolute contraindication. A simple base line of blood and urine can be completed within 48 hours and treatment instituted promptly, when it is usually most effective.

Of the 10 patients on iproniazid, in from 1 to 7 days suicidal symptoms were relieved in 5, 3 showed no change, and 2 became worse. The 3 who showed no change were put on RO 4-1018 and showed no improvement. RO 4-1018 seemed slightly less rapid and potent in this group although, in the chronic female psychotics, it seemed slightly more so.

Of the 5 suicidal patients started on RO 4-1018, 1 improved rapidly, 1 slowly and, in the other 3, there was only minimal response.

The dose of both iproniazid and RO 4-1018 was 50 mg. 3 times daily. Those who refused to take oral medication were given 50 mg. once a day by injection until they improved sufficiently to take the oral medication, also at the same level. We again emphasize that, once the patient starts improving, it is good clinical practice to reduce the dosage, in this case by approximately one half each week until it reaches 10 mg. daily for iproniazid and 10 mg. twice daily for RO 4-1018.

Those patients who responded with hyperexcitability were all cases of agitated depressions, but there also were many patients with agitated depressions who responded adequately without the induced hyperactivity. When adjunctive medication was added subsequently, as a rule the patients were brought under control. We have already stressed the superiority of psychic energizers in the treatment of acute suicidal patients, and RO 4-1018 appears to belong in this class. In general, we recommend that for schizophrenics it be used in combination with an ataractic. Although there were cases of improvement in the area of psychopathology on the psychic energizers alone, results were better when they were used in combination.

Sixteen other patients characterized by depression, apathy, or withdrawal were treated with RO 4-1018. Nine of these showed great improvement and have been or are to be discharged. Five failed to show significant change and, in 2 cases increasing hyperactivity clinically worsened the patients until it was necessary to withdraw medication. Two patients developed mild jaundice. One of these showed no clinical response to either drug, but while on iproniazid he developed an ecthymalike skin eruption that subsided after withdrawal. With RO 4-1018 he manifested subclinical jaundice with an icteric index of 16, which rapidly returned to normal after discontinuance. The second patient exhibited gratifying and rapid psychiatric improvement. With the yellowed sclera he had a fever of 103° F., an elevated white blood count, and an icteric index of 20. All of these symptoms subsided after withdrawal of medication, but his mental condition regressed.

A later patient in an RO 4-1018 series became hyperactive, showing an icteric index of 16 without evident jaundice and a paradoxical hypertension of 180/110. After cessation of medication, agitation lessened, hypertension disappeared, and the icteric index returned to normal. In this case the clinical picture indicated that jaundice was due to infectious hepatitis.

Two patients who developed hypotensive effects were completely relieved by reduction of dosage. In one the frequency of previous anginal attacks was reduced to zero; in the other there was sporadic difficulty with both delayed and involuntary micturition. One 70-year-old semirigid involutional melancholic, absolutely unmoved by doses as high as 200 mg. (50 mg. four times daily) of RO 4-1018 after 5 months of intensive treatment, is currently showing definite affective improvement with 50 mg. four times daily of imipramine (Tofranil, G-22355). In 5 patients who showed no significant improvement on RO 4-1018 alone, the results were generally more effective when phenothiazines were added. It is felt that, if the ataractic drug dosage were further increased, even more favorable results would be achieved. We therefore recommend on clinical grounds that individual consideration be given to the use of a psychic energizer alone or in combination with an ataractic. Patients manifesting strong anxiety or agitation should be particularly considered for combined treatment.

Ten chronic schizophrenic females, aged 36 to 54, hospitalized for from 5 to 25 years, were selected because they were passively cooperative, idle, seclusive, withdrawn, apathetic, and preoccupied. Treated with a daily morning dose of 50 mg. of RO 4-1018 for 3 months, 2 became more interested in their surroundings and definitely more alert, one remained unimproved, and 7 became clinically more difficult because of anxiety, agitation, increased restlessness, irritability and, in 2 cases, actual assaultiveness. The schizophrenic psychopathological thinking was not altered. Dosage was then reduced to 25 mg. a day in the 2 patients who had shown improvement and to 10 mg. a day in the 7 overactive ones. There was now a marked lessening of the intensity of agitation, although it did not entirely disappear in all patients. Instead of the expected weight increase, most of the patients lost from 4 to 14 pounds during the 3-month period. During the first 2 weeks 2 patients developed nausea and vomiting, which subsequently subsided. After 2 months one patient's BUN rose from a pretreatment level of 17.5 mg. per cent to over 32 mg. per cent, but subsided to normal 2 weeks after the withdrawal of medication. Another patient's hemoglobin dropped to 10 gm. and his red blood count (RBC) to 3.45 million, but there was no shift in the differential, and the white blood count remained between 4000 and 8000. After withdrawal of medication, blood indices returned to normal.

In summary, iproniazid and RO 4-1018 are roughly comparable in effectiveness and, in patients who fail to respond to iproniazid, there would be justification in using RO 4-1018 as an alternate medication. We did not note edema with the newer preparation. There were side effects in a few patients, but a larger series of such reactions must be investigated to determine their relative incidence compared with those attributed to iproniazid. The effects were of the same type as those found with iproniazid, and the percentage of patients affected falls within the realm of statistical probability.

(4) *RO 5-0700* (*Roche*). One half of the 16 female patients in 1 subgroup had been hospitalized for 3 years or less, the other half 3 years or longer, all with depression, lack of interest, apathy, or similar symptoms. As of this writing they have been treated for slightly less than 2 months on 10 mg. three times daily. Three of those hospitalized less than 3 years have already shown marked improvement, 2 of them appearing to be ready for discharge. No side effects were noted in any of the 16. One patient who occasionally goes into cataleptic episodes also did so on this medication, but is now responding to the addition of perphenazine (Trilafon). Other clinical data on RO 5-0700 are not sufficient to report at the present time.

Side Effects

Certain side effects may be roughly related to dosage but, as a rule, they are not a function of it. More frequently, highly sensitive individuals react to minute doses, whereas more resistant patients appear to be almost immune to side reactions. One patient, in a suicide attempt, swallowed 50 of the 25-mg. iproniazid tablets at one time. The 1250 mg. produced slight dizziness and weakness, and the only other effect was that the patient "felt pretty good." This dose, however, was almost homeopathic compared with that of a patient in Calcutta, India, who was taking 150 mg. daily when his doctor left on a 4-month assignment to another part of the country. The patient then began to increase the dosage until at the end of 2 months, on 800 to 1000 mg. daily, he wrote to complain of difficulty in urination and "sexual impotence." The dose is now back within the terrestrial range; not only does the patient feel better, but the side effects have disappeared.

Side effects tend to be quite similar for all of the amine oxidase inhibitors. The apparent differences in our series may be due in part to the relatively small number of cases, but we feel that they should be noted. Dizziness, ataxia, and feelings of weakness resulting from orthostatic hypotension occurred in only a few of the iproniazid patients and were even less frequent with the newer compounds. A patient who responds to one amine oxidase inhibitor with this particular side effect will almost invariably give a similar response to the others, although it may take longer to occur; reduction of dosage and the addition of an amphetamine are usually sufficient to handle it. Both periorbital and dependent edema have been noted to occur with each of the new psychic energizers. It is usually adequately managed by reduction in dosage or adjunctive therapy; in no case has it been necessary to discontinue treatment.

Three patients on the newer compounds have shown presumptive evidence of liver disorder as manifested by 2 cases of subclinical and one of clinical jaundice but, in at least one case, the diagnosis was probably infectious hepatitis. Since the evidence of jaundice is so low, even with ipronaizid, no comparison can be made until at least several thousand cases have been treated on each compound. Other occasional side effects would include constipation, delayed micturition, impotence (and its opposite), pruritus, gastritis, anemia and, in one case, uremia (in a patient given RO 4-1018). In roughly 70 per cent of the patients there is a recrudescence of psychopathology accompanied by hyperactivity, irritability, agitation and, at times, assaultiveness: reduction of dosage and the addition of phenothiazine derivatives are definitely indicated. There are occasional neuromuscular symptoms including flaccidity, paresthesias, and twitching and, in a rare case, evidence of neuralgia. In these instances we recommend the use of pyridoxine hydrochloride (vitamin B_6) in doses up to 50 mg. daily. There is generally an increase in weight, with the exception noted above. This is accompanied by a positive nitrogen balance (where it has been tested), so that the weight increase is more than an accumulation of fluid. There may be occasional paradoxical fatigue and sleepiness, perhaps resulting from initial overdosage, but this has not been definitively determined.

Of particular importance is the fact that some amine oxidase inhibitors potentiate other medications, including adrenaline and the caffeine found in coffee, tea, or Coca Cola.

The patient should always be told that there may be a reduced need for sleep, so that he does not medicate himself or have his local physician oversedate him. Some show no fatigue on only 2½ to 3 hours sleep nightly, and consequently they can carry out many more activities than before. Others, with long histories of insomnia, paradoxically increase their sleep for the first time in many years. Although the sleep may last no more than 4 or 5 hours, it is usually sound, in contrast to the previous napping and tossing over periods of 10 or 12 hours.

In a number of cases thyroid extract (for whatever reason) does not seem to combine well with the amine oxidase inhibitors and may produce sluggishness and apathy. This is an interesting lead for investigation of the mode of action of both compounds. Where, after prolonged usage, or even more frequently after intermittent usage, the initial increase in activity is not maintained, the addition of a protein hydrolyzate will renew the drugs' effectiveness. Barbiturates, as well as other medications, may be used and, occasionally, should be used in combination with the psychic energizers; as a rule, however, lower doses of the former are definitely indicated.

Combinations of Drugs

As amply illustrated in the specific results already cited, there is strong evidence that the addition of an amphetamine or an amphetaminelike compound will expedite the initial reaction and will help to counteract orthostatic hypotension in those cases in which it appears. In patients with agitated depressions, a combination of an amine oxidase inhibitor with a phrenotropic agent (also used at a lower-than-usual dose) is extremely valuable. Certainly, in the treatment of schizophrenics, the phenothiazine derivatives or *Rauwolfia* alkaloids are necessary to correct the psychopathology, whereas the psychic energizers may stir the patients sufficiently to participate in various activities. The action of imipramine (G-22355, Tofranil) is often valuable either in combination or subsequent to the use of amine oxidase inhibitors. The amine oxidase inhibitor relieves the depression but, at times, the patient is left with residual problems that consume a large part of his energies. We feel that the antidepressant and psychostimulating action of imipramine results from a release of energy otherwise bound in neurotic or psychotic processes. We also feel that this drug has actions other than antidepressant ones so that its spectrum of activity may be considerably broader than is presently thought. On the other hand, there are cases that fail to respond to months of treatment with imipramine but subsequently respond rapidly and completely to one of the psychic energizers. Clarification of these points should also go far to improve our diagnostic and taxonomic abilities. The patients with purely affective disorders of the "essential" type respond best to the amine oxidase inhibitors, whereas those with schizophrenic or severe neurotic conditions with secondary depression respond better to imipramine or combinations of energizers and phenothiazines.

Summary and Conclusions

(1) Four new amine oxidase inhibitors were tested to determine whether they possess the same psychic energizing effect as iproniazid. In every case it was found that there were areas of similarity in psychic action. This appears to establish firmly the principle that pharmaceuticals that act as amine oxidase inhibitors are capable of increasing activity or relieving depression.

(2) Side effects such as orthostatic hypotension in some patients are common to all of the psychic energizers, but differences appear in some of the minor side effects. It has also been noted that in some patients side effects are more frequent on 1 preparation than on another so that, if these should continue following dosage adjustment to the extent where they become an impediment to treatment, the patient should be transferred to a different psychic energizer.

In the present series, side effects including elevated BUN, clinical jaundice, elevated icteric index, uremia, and drop in hemoglobin and RBC were noted in 3 patients on RO 4-1018. The other psychic energizers produced relatively few side effects.

(3) On the doses used there was no significant difference in effectiveness between any of the psychic energizers although, at times, one patient would respond better to one drug than to another. In one case at least, a private patient distinguished between a "festive energy" produced by W-1544 and the "internal energy," which did not result in her socializing, as produced by two other energizers. RO 4-1018 was felt to be more active with chronic patients than iproniazid, but somewhat less effective in the acute patient.

(4) In a group of children and adolescents receiving one half the usual dose of JB-516, it would appear from the accumulated evidence that an adult dose would be desirable.

(5) There is evidence that the combination of an amphetamine or amphetaminelike compound with the psychic energizer will expedite the time of reaction.

(6) Almost without exception, patients with "essential" depressions responded favorably to psychic energizers. Patients whose depression was secondary to a psychotic or a severe neurotic process seemed to respond better to a combination of a psychic energizer with an ataractic or to imipramine (Tofranil).

(7) In the treatment of agitated depressions it is suggested that an ataractic be used in combination with the amine oxidase inhibitor if complete relief of symptoms is not obtained. This does not preclude the use of an amphetamine or amphetaminelike compound as well.

(8) In the treatment of schizophrenics with depression, apathy, and withdrawal, the psychic energizers almost without exception effected a change When used alone, they produced a definite improvement in approximately 25 per cent of the acute patients but a worsening in 75 per cent, shown in increased hyperexcitability, hyperactivity and, at times, assaultiveness, with aberrant thinking. If, initially in the treatment of the acute schizophrenic, an ataractic was used in combination, approximately one third of the patients were markedly improved, another one third showed some improvement in their psychosis and the remainder showed over-all clinical worsening.

In the treatment of the chronic schizophrenic approximately two thirds o the patients showed a definite change if iproniazid is used alone: approximately one half improve, the balance worsening. Therefore, favorable results can b obtained by the use of an ataractic in combination with the psychic energizers

(9) The idea that a principle of drug action rather than a chemical structure is involved points the way toward investigation of mode of action. This concept may be of greater importance than the therapeutic benefits of this new group of compounds.

(10) The advantages of psychic energizers over electroshock therapy in th treatment of depressed and suicidal patients are discussed.

(11) Psychic energizers potentiate many other medications. However W-1544 appears to have little if any potentiating action or effect on the bar

biturates. The combination of thyroid preparation or cocaine derivatives with the psychic energizers appears to be contraindicated.

(12) In schizophrenics treated with ataractics who have reached the "plateau" of secondary symptoms with continuance of primary ones, the addition of psychic energizers often will cause a change. The same is true of patients with seemingly fixed symptoms of long duration where a shift may occur in a favorable direction, although at times a clinical worsening may be produced.

(13) We recommend high initial doses and continued use of these medications until response has been obtained, after which dosage should be reduced rapidly until it can be eliminated or a maintenance dose established.

(14) Since the drugs are of a potency comparable to that of digitalis, great care must be exercised in the titration of dosage to meet the needs of each patient.

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References

1. LOOMER, H. P., J. C. SAUNDERS & N. S. KLINE. 1957. A clinical and pharmacodynamic evaluation of iproniazid as a psychic energizer. Am. Psychiat. Assoc. Psychiatric Research Repts. **8:** 129.
2. BLEULER, E. 1950. Dementia Praecox, or the Group of Schizophrenias. J. Zinkin, Translator. Intern. Univs. Press. New York, N. Y.

Discussion of the Paper

ALEXANDER P. DUKAY (*Ypsilanti State Hospital, Ypsilanti, Mich*): Without hesitation, one may describe the last decade in psychiatry as the third revolution in that discipline.

The first of these is connected with the name of Philippe Pinel, of an era of social reform and descriptive psychiatry; the second with the name of Sigmund Freud, the concepts of deterministic materialism and psychodynamics. The third one, opening the era of psychopharmacology with advances in neurochemistry and pharmacology, is associated with many of the names that appear on the cover of this monograph. Kline and his co-workers have presented another of their many contributions to this field.

In our experience with about 100 female chronic hospitalized psychotic patients, amine oxidase inhibitors were without serious side reactions. Orthostatic hypotension was the most frequent and burdensome of them, persisting 5 to 10 days after the withdrawal of the drug. Ankle edema and erythema were next in frequency.

Iproniazid (Marsilid) brought about definite improvement in one third of our patients; one third remained without change. Patients classified according to depressive symptomatology in one group improved on one compound after being refractory to a similar one. Obviously, we are dealing with a heterogeneous group of human beings whose common characteristic is their overt behavior of depression, psychomotor retardation, and anxiety, with amine oxidase levels as a common physiological characteristic. The above-described common characteristic is the final common path for the complex multilevel and multidimensional interaction between biological, genetic, neurohumoral, socioeconomic, and cultural forces. Any attempt to discover causal relationships between enzyme levels, drug-specific inhibitory potency, and success in treatment as reflected only in change in overt behavior is doomed to disappointment.

One might easily find himself in the position of one of our very enthusiastic ward supervisors who, participating in the pilot Marsilid investigation[1] under the direction of a devoted and spirited psychiatrist, discovered on the previously enigmatic faces of her patients the Mona Lisa (Marsilid) smile. This supervisor also discovered the Marsilid smile on her patients during a later double-blind study, only to find out that her patients were on placebos. This experience is not unique. We have read in recent psychiatric literature about unusual treatment successes.[2] The investigator in question created a nickname, the Widow's Delight, for one of the amine oxidase inhibitors.

Therapy in medicine has made astonishing progress during the last two decades. If we in psychiatry do not want to remain antiquated, we must follow its path. To illustrate this contention, an analogy from the medical field may be made:

A uniform group classified according to symptoms, namely, fever and cough, given streptomycin over a period of months, would be subdivided according to therapeutic responses into at least 3 groups: the first, with dramatic improvement; the second, with some improvement; and the third, without any change.

By subjecting the first subgroup (established according to drug response) to multidimensional investigation, such as chest X rays, sputum studies, tuberculin tests, and clinical examination, we certainly should discover the causal relationships between fever, cough, Koch's bacillus, and streptomycin.

I have great hopes that the amine oxidase inhibitors and other psychoactive drugs will enable us to separate from the large heterogeneous group of similar psychiatric symptomatology smaller, well-defined entities according to drug responses and help us to establish causal relationships.

We have set out on this road, as few other centers in this country have, in a joint project with the University of Michigan at the Ypsilanti State Hospital. This involves intensive investigation of relatively few patients by biochemical, physiological, psychological, and psychiatric methods in an attempt to find intercorrelations between enzyme levels, inhibitor potency, polygraph patterns, overt behavior, psychodynamics, and drug responses.

Amine oxidase inhibitor research serves as a very useful theoretical model into which we have to fit the bricks of biochemical and psychiatric observations to obtain a meaningful unity. The presentation of Kline and his co-authors is an effort toward this goal.

References

1. Dart, D. & A. P. Dukay. 1957. A clinical experience with Marsilid. Presented a semiannual meeting, Michigan State Hospital and Clinic Physicians' Association, Oct
2. Robie, T. S. 1958. Iproniazid chemotherapy in melancholia. Am. J. Psychiat. **11** (5): 402–409.

THE INERT PSYCHASTHENIC REACTION (ANHEDONIA) AS DIFFERENTIATED FROM CLASSIC DEPRESSION AND ITS RESPONSE TO IPRONIAZID

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The development over the past twenty years of physical treatments with a wide range of effectiveness, due probably to the fact that they all reduced the general excitability of the higher nervous activity of the cortex,[1] has tended to blur the outlines of the nosological entities that had been painstakingly drawn during the preceding one hundred years. However, the recent advent of drugs with rather specific effects on symptomatology is forcing us to renew our interest in the finer differentiations of clinical nosology with an eye, perhaps, toward uncovering brain mechanisms that may be specifically influenced by the newer and more highly specialized drugs.

The fact that the new drugs relieve specific aspects of symptom formation rather than broader categories of illness has been perceptively stated by Kalinowski.[2] Freyhan[3] speaks of effects upon specific "target symptoms" as the mode of action of drug therapy. To this we should like to add the concept of "key symptoms"; if the target symptom also happens to be a key symptom of primary importance in evoking a chain of other reactions that constitute the clinical picture, the entire remainder of the clinical disturbance may be relieved as well, in line with Gantt's[4] concept of autokinesis.

Quite apart from its traditional division into manic-depressive, involutional, and neurotic depressions, depression is not a nosologically uniform symptom complex, but may represent strikingly different clinical syndromes that cut across the dividing lines named above. There are depressions in all of these groups that lack the classic symptoms of remorse and self reproach, but instead exhibit inability to experience pleasure and satisfaction, a joyless inhibited state that Myerson[5] called anhedonia. Instead of self-reproaches, these patients abound with self-pity. They do not consider themselves wicked, but merely weak and powerless. They are not driven to madness by the furies of remorse like Orestes but, like Job, they wallow in self-pity: "Why does this happen to me, a good man?" These patients are often characterized by profound inertia and psychomotor inhibition. The most severe cases of this type come close to or include Hoch's[6] "benign stupors" and Kleist's psychomotor depressions.[7]

Rosen and one of us (L.A.),[8] in studying the psychological issues that were operative in depressions, found that the impelling issue in this type of case is envy, in contrast to the issue of guilt operative in the classic depressions. In terms of Bibring's[9] narcissistic aims, the dynamic narcissism in these cases is not the need for moral goodness or worthiness, but the need for power that appears to be frustrated.

We should like to call this depressive syndrome the inert psychasthenic-anhedonic reaction. We have chosen the word "psychasthenic" to emphasize the importance of weakness and powerlessness, and also to indicate the relationship to the other psychasthenic reactions first established by Janet,[10] namely, to the phobias and compulsions that some aspects of these patients' symptomatology suggest.

Characteristic complaints among the neurotic depressions of this type are inability to do anything—to work or even to go out of the house—fatigue, exhaustion, listlessness, not feeling well, unhappiness, being "down," inability to eat, diffidence, and lack of self-confidence. Self-reproaches are entirely absent. When sleep disturbance is a complaint, it is usually in terms of difficulty or inability to fall asleep; only one patient complained of early waking, none of frequent waking at night. Two patients complained of sleeping excessively, night and day.

Among psychotic patients of this type, such complaints are similar, however with more emphasis on inadequacy, loss of confidence, unsureness, physical inability, fear of doing anything (in one case even of getting into the bathtub), incapability of feeling emotion, uncertainty, doubts, weakness, and suicidal trends. Self-reproaches are absent as in the neurotic group. However, sleep disturbance is more severe, including difficulty in falling asleep, frequent waking at night, and early waking in the morning, in about one half of the cases. In these psychotic cases the clinical picture is further complicated by behavioral disturbances, especially profound psychomotor inhibition, sometimes of almost catatonoid degree, which render the proportion of the total picture psychotic and call forth the usual protective measures necessary for the care of the psychotic patient.

Rosen and one of us (L.A.)[8] had observed for some time that depressed patients with this psychasthenic-anhedonic symptomatology responded less well to convulsive electroshock than did the patients with the classic symptoms of depression in that they tended to achieve less full recoveries and to relapse frequently. We found that these patients responded relatively better to low-amperage convulsions at minimum threshold level than to standard electroshock. However, even thus treated, many of these patients remained a problem with chronic or frequently recurring illness; a few spectacular exceptions responded in a sustained manner to low-amperage treatment.

Case Material

Our present study is based on 54 consecutive cases of depression, including all types, whose treatment was initiated between June 1957 and January 1958. Evaluation of the results is based on continued follow-up study until October 30, 1958.

Method of Treatment

In all but 3 patients, iproniazid (Marsilid*) therapy was started at the dosage of 50 mg. orally 3 times daily, as originally recommended by Kline and his co-workers.[11] In 3 patients the initial dosage was 25 mg. 3 times daily. All patients concurrently received pyridoxine, 50 mg. orally daily.

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* Hoffmann-La Roche.

As soon as a favorable treatment result was obtained, the initial dosage was gradually reduced, the first step being a reduction to 50 mg. twice daily or to 25 mg. 3 times daily, depending on the amount of improvement. In the patients who recovered, the initial high dosage was administered for from 1 to 12 weeks, the average being 5 weeks. It turned out that the duration of administration of the initial dosage for patients who failed to recover was practically identical, namely, from 1 to 17 weeks, the average being also 5 weeks.

After another period averaging 5 weeks, the dosage was again revised downward, whenever possible, that is, consistent with maintenance of the gains achieved. After further gradual reduction of the dosage over a subsequent period averaging 12 weeks, it was possible either to discontinue the medication or to reduce it to a maintenance dose of as little as 10 mg. every other day. Some patients, however, required maintenance doses as high as 50 mg. daily (4 patients) or even 50 mg. twice daily (one case), since relapse took place whenever the dosage was lowered below this level.

In 9 of the 11 patients in whom serious side effects developed it was necessary to discontinue the medication; in 2 of them these side effects yielded to lowering of the dosage used.

As to duration of treatment, the following facts emerged: of the 24 successfully treated patients, it was necessary to keep 11 on medication for the entire period of observation, this period averaging 45 weeks. The maintenance dosages required for these patients varied from 50 mg. twice daily to 10 mg. every other day, the average being 25 mg. daily. Only 13 of the 24 recovered patients were able to terminate treatment prior to expiration of the observation period, the duration of the treatment averaging 22 weeks. Treatment failures remained on the drug from 4 days to 55 weeks, the average being 15 weeks. (It was necessary to discontinue the case treated for only 4 days because of jaundice; this case has been reported in detail by Zetzel and Kaplan.[12] Two months previously, she had been on the drug for 8 days without ill effects.)

Criteria for Treatment Results

As criteria for the evaluation of treatment results, we utilized the definition for grades of improvement adopted by one of us some time ago.[13] Complete recovery is defined as full social and occupational rehabilitation, as well as restoration of the mental state of the patient to what he and his relatives agree is his former "own best self." This implies that full recovery is associated with insight, although it may not be entirely lacking in other degrees of improvement.

A social remission or social recovery describes a patient who has achieved full social and occupational rehabilitation, but who has either failed to achieve insight or still retains some subjective symptoms or complaints in his intimate personal life.

These two groups of patients are pooled as the recovered group. A further condition for the inclusion of a patient in the recovered group is that this state of recovery be maintained for at least six months after it has been first established and that no other physical treatment be necessary during that period.

The nonrecovered group consists of patients who either remained unimproved, or who improved short of social recovery, or who relapsed within six months or less.

Results in all Cases

The recovery rate on iproniazid for the entire group was 44 per cent of 54 cases. Viewed against the background of the outcome of other therapies, this recovery rate is statistically not significant in comparison with a group treated with psychotherapy alone,[1] in which the recovery rate was 44 per cent of 25 cases. Another group treated with electroshock therapy showed a recovery rate of 73 per cent of 76 cases.[14] This rate differs significantly from that of the iproniazid cases ($\chi^2 = 10.3$, $P = .001$) and from the psychotherapy group ($\chi^2 = 6.7$, $P = .01$).* The frequencies of recovery for the 3 treatments are presented in TABLE 1. For these figures χ^2 is 12.7, which is significant below the 1 per cent level, indicating that the several treatments do differ in their effectiveness.

TABLE 1

	Iproniazid	Psth*	ECT†	Total
Recovered	24	11	55	90
Not recovered	30	14	21	65
Total	54	25	76	155

$\chi^2 = 12.66$
$P < .01$

* Psychotherapy.
† Electroconvulsive therapy.

TABLE 2

	No.	Per cent recovered	χ^2	P
Iproniazid	54	44	.67	.41
Psth*	25	44	.36	.55
ECT†	76	73	15.20	<.001

* Psychotherapy,
† Electroconvulsive therapy.

When we consider these treatments separately with respect to the questio of what proportions recovers under each, we find that only with electroshock therapy does a significantly greater proportion of cases recover. These figures are presented in TABLE 2.

Neurotic, as compared to psychotic depressive reactions. However, when th iproniazid-treated patients are separated into neurotic (25) and psychotic (29) depressions, it appears that 64 per cent of the neurotic patients, but only

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* The recovery rates for both the psychotherapy and electroshock therapy groups were representative of those established for larger groups collected from various sources in the literature[13] (46 per cent of 208 cases treated with psychotherapy and 68 per cent of 2165 cases treated with electroshock therapy recovered).

28 per cent of the psychotic patients, recovered on iproniazid. This difference in percentages is significant below the .01 level ($\chi^2 = 7.2$; see TABLE 3).

Psychasthenic-anhedonic, as compared to classic depressive reactions. A still sharper differentiation was obtained when the group of depressive illnesses treated was separated into the psychasthenic-anhedonic reactions (16) and the depressive reactions of classic symptomatology (38). The recovery rate on iproniazid was 88 per cent for the psychasthenic-anhedonic reactions, while it was only 26 per cent for the classic depressive reactions. This difference is significant below the .001 level ($\chi^2 = 17.1$; see TABLE 4). This high recovery rate was the same for those psychasthenic-anhedonic reactions that had been classified as psychotic as it was for those classified as neurotic, the recovery rate being 7 out of 8 (88 per cent) for either group (TABLE 5).*

TABLE 3
IPRONIAZID

	Recovered	Nonrecovered	Total
Neurotic	16 (64%)	9	25
Psychotic	8 (28%)	21	29
Total	24 (44%)	30	54

$\chi^2 = 7.2$
P < .01

TABLE 4
IPRONIAZID

	Recovered	Nonrecovered	Total
Psychasthenic	14 (88%)	2	16
Classic	10 (26%)	28	38
Total	24 (44%)	30	54

$\chi^2 = 17.1$
P < .001

By contrast, when the depressions with classic symptomatology were subdivided into psychotic and neurotic ones, it appeared that of 21 psychotic depressions with classic symptomatology only 1 recovered, while 20 failed to recover. One of these, who suffered from a severe suicidal depression and who had made one suicidal attempt before referral, made 2 further such attempts while on iproniazid, the second one in hospital after 34 days on iproniazid medication at the dosage of 150 mg. daily. However, of the 17 neurotic depressions of classic symptomatology, 9 recovered, while 8 did not. These differences within the classic depressive reactions are significant below the .001 level ($\chi^2 = 11.2$; see TABLE 6).

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* In view of the relationship of the psychasthenic depressive syndrome to compulsive-obsessive states and phobias, it may be of interest that in one severe obsessive-compulsive state with schizoaffective and catatonic features that had been unresponsive to other forms of intensive treatment, including analytic psychotherapy, several courses of electroshock therapy, and a course of insulin coma therapy (60 deep comas) achieved a spectacular state of full recovery on iproniazid. Two cases of phobias, however, were not improved to the point of social recovery.

These findings suggest that psychasthenic reactions may be treated effectively with iproniazid regardless of neurotic or psychotic symptomatology, while classic depressive reactions may not successfully be treated with iproniazid in the presence of psychotic symptomatology. However, these conclusions should be tempered by the fact that, if both classifications—psychotic versus neurotic and psychasthenic versus classic—are taken into consideration simultaneously, no particular combination of these factors is significant; that is, psychasthenic patients are better prospects for iproniazid therapy than ar those with classic depressions, and neurotic patients are better than psychoti ones. However, if the depression is of psychasthenic type, psychotics an neurotics are equally good prospects while, in the case of classic depressions psychotics and neurotics are equally poor prospects, since even in the neuroti subgroup the treatment successes with iproniazid do not significantly outnumber the treatment failures.

TABLE 5
IPRONIAZID

Psychasthenic reactions

	Recovered	Nonrecovered	Total
Psychotic	7 (88%)	1	8
Neurotic	7 (88%)	1	8
Total	14	2	16

$\chi^2 = 0$

TABLE 6
IPRONIAZID

Classic depressive reactions

	Recovered	Nonrecovered	Total
Psychotic	1 (5%)	20	21
Neurotic	9 (53%)	8	17
Total	10 (26%)	28	38

$\chi^2 = 11.2$
$P < .001$

*Patients with past history of electroshock failure.** In view of the observation made on clinical grounds as referred to above, that those patients did relativel best on iproniazid who had done relatively poorest on electroshock therapy, we made a breakdown of iproniazid-treated cases into those who had on previous occasions responded with recovery to electroshock therapy (7), those who had failed on electrochock therapy before (24), and those who had no previous electroshock therapy (23). These 3 groups of patients are not significantly differentiated with respect to success or failure of iproniazid treatment ($\chi^2 = 1.6$, $P = .20$).

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* Electroshock therapy failure is defined as the situation in which a patient who, followin electroshock therapy, either remained unimproved or improved short of social recovery or wh relapsed within 6 months or who, on 3 or more successive occasions, relapsed within less tha 1 year.

Of particular interest are those patients who previously were electroshock failures. Of the 24 such patients, 11 showed psychasthenic-anhedonic symptomatology, and 13 showed symptoms of classic depression. In the first group, 9 recovered on iproniazid while 2 did not; in the second group, only 3 recovered while 10 did not (TABLE 7). This difference is significant below the .02 level ($\chi^2 = 6.0$ with Yates' correction).

In summary, electroshock failure in the past does not indicate favorable or unfavorable prognosis for treatment with iproniazid per se. However, in the psychasthenic group, electroshock failure in the past does not detract from the favorable chances of treatment outcome with iproniazid, while in the classic depressions it does not alter the unfavorable prospects.

TABLE 7
PREVIOUS ECT FAILURES

	Iproniazid		
	Recovered	Nonrecovered	Total
Psychasthenic	9 (82%)	2	11
Classic	3 (23%)	10	13
Total	12 (50%)	12	24

χ^2 (with Yates' correction) = 6.0
$P < .02$

Patients differentiated by adrenaline-mecholyl test pattern.* We also broke down the cases by Funkenstein's test pattern,[15, 16] distinguishing Funkenstein's favorable group (Types VI–VII) from his unfavorable group (Types I–V). Funkenstein's terms "favorable" and "unfavorable" apply to their predictive outlook for electroshock therapy. The predictive value of the test for the outcome of electroschock treatment has been confirmed in the literature (Moriarty,[17] Rouleau and Coulombe;[18] Alexander[1]).

In iproniazid treatment, 5 of 15 Funkenstein-favorable cases recovered (33 per cent), while 7 of 14 Funkenstein-unfavorable cases recovered (50 per cent). This difference is not significant, but it is consistent with our findings with respect to the lack of predictive relationship between the outcome of previous electroschock therapy and outcome of iproniazid therapy.

It may be worth mentioning, however, that 2 of 4 Type V cases in our group of iproniazid-treated patients recovered. This is of interest in that Type V

* Acetyl-beta-methylcholine (Merck).

cases are notoriously treatment-resistant and constitute the core of the chronic incurable cases.

Side Effects

Complications and side effects were numerous. Serious side effects occurred in 11 (20 per cent) of the patients treated. They included 1 death from an extensive cerebrovascular thrombosis in a woman of 71 years. There were 3 cases of liver damage with jaundice (all of them recovered, one with the aid of ACTH therapy), and 2 cases who developed a hyperinsulinism-like state that was considered as possibly due to occult liver damage characterized by failure of glycolysis. One of these also developed multiple sclerosis-like neurological signs and symptoms, with increase of the spinal fluid protein.

Four patients developed neurocirculatory disturbances described by the various patients as dizziness, faintness, fainting spells, blackouts, blurred vision, inability to focus, and darkening of vision (greyout). These symptoms, obviously related to cerebral anoxia, were not always correlated to the degree of hypotension observed. In the 3 most severe cases of this type, hypotension was relatively slight, blood pressures measured in the sitting position having declined only from 168/96 to 124/70, 138/88 to 106/74, and 110/70 to 96/60, respectively. At the same time a patient with a more marked fall in blood pressure (from 120/74 to 72/50 in the erect-sitting, and standing positions, and to 90/60 in recumbent position) remained free from subjective symptoms or other side effects.

In 2 of the patients with neurocirculatory side effects, lowering of the dosage relieved the symptoms while maintaining the patient in the state of social recovery that had been achieved. In 2 others, discontinuance was necessary. One of these retained her state of full recovery, while the other remained unimproved. One patient developed impotence and intractable constipation, requiring discontinuance of the drug.

Minor side effects were dry mouth, aching back muscles, muscular twitching, and constipation, which yielded promptly to lowering the dosage.

On the basis of our experience, we adopted the following protective routine in addition to the administration of 50 mg. of pyridoxine daily, which had been carried out from the beginning, namely, liver function tests, and evaluation by a specialist in internal medicine prior to treatment, once weekly during the first month of treatment, bi-weekly during the subsequent 3 months, and once monthly thereafter. Tests used are Bromsulphalein, transaminase, cephalin flocculation, and serum bilirubin.

Psychotherapeutic interviews are held once weekly during the first 3 months of treatment and every other week thereafter. On these occasions, the blood pressure is determined and the tendon reflexes evaluated. If the blood pressure is found to have declined excessively or the tendon reflexes are found to have become excessively brisk or hyperactive, the dosage is lowered independent of psychiatric indications. This, of course, is also done whenever subjective side effects appear. Constipation is avoided throughout by prescribing a sufficiency of milk of magnesia before bedtime, up to 4 tablespoons daily. The importance of this measure has been stressed by S. Joel (personal communication).

Summary

Iproniazid offers specific relief for a depressive syndrome best described as psychasthenic-anhedonic reaction, while it is not of significant benefit for the classic depressive reactions.

Iproniazid may accomplish this selective effect because its target symptom, the feeling of weakness and powerlessness, is of crucial importance as a key symptom in the psychasthenic-anhedonic reactions, but it is not of significant dynamic importance in the classic depressive reactions.

The side effects encountered in the use of iproniazid should not discourage its use in the hands of a psychiatrist aided by an internist with adequate clinical facilities at their disposal, but should emphasize the importance of specific indications for its use and careful and attentive management of the patient through all phases of administration of the drug.

A severe psychasthenic-anhedonic reaction, neurotic or psychotic, after electroshock failure may constitute the most significant indication for iproniazid therapy. All other indications are relative, the benefits expected to be weighed against the risks incurred.

References

1. Alexander, L. 1958. Objective Approaches to Treatment in Psychiatry. I–VIII: 1–139. Thomas. Springfield, Ill.
2. Kalinowsky, L. B. 1958. Appraisal of the "Tranquilizers" and their influence on other somatic treatments in psychiatry. Am. J. Psychiat. **115:** 294–298.
3. Freyhan, F. A. 1956. L'Encephale. **4:** 913.
4. Gantt, H. W. 1953. Principles of nervous breakdown: Schizokinesis and autokinesis. Ann. N. Y. Acad. Sci. **56**(2): 143–163.
5. Myerson, A. 1922. Anhedonia. Am. J. Psychiat. **2:** 87–103.
6. Hoch, A. 1921. Benign Stupors. MacMillan. New York, N. Y.
7. Kleist, K. 1925. Die gegenwärtigen Strömungen in der Psychiatrie. Allgem. Z. Psychiat. **82:** 1–41.
8. Alexander, L. & I. Rosen. 1955. Management of psychological issues in conjunction with physical treatment. Diseases of Nervous System. **16:** 232–236.
9. Bibring, E. 1953. The mechanism of depression. *In* Affective Disorders, Psychoanalytic Contribution to their Study. P. Greenacre, Ed. : 13–48. Intern. Univs. Press. New York, N. Y.
10. Janet, P. 1929. The Major Symptoms of Hysteria. MacMillan. New York, N. Y.
11. Kline, N. S. 1958. Clinical experience with iproniazid (Marsilid). Clin. Exptl. Psychopathol. **19:** 72–78.
12. Zetzel, L. & H. Kaplan. 1958. Liver damage concurrent with iproniazid administration. New Engl. J. Med. **258:** 1209–1211.
13. Alexander, L. 1953. Treatment of Mental Disorder. i–xi. : 1–507. Saunders. Philadelphia, Pa. & London, England.
14. Alexander, L. 1954. Outpatient electroshock therapy in psychoses. The Medical Clinics of North America. **38:** 1363–1376. Saunders. Philadelphia, Pa.
15. Funkenstein, D. H., M. Greenblatt & H. C. Solomon. 1952. An autonomic nervous system test of prognostic significance in relation to electroshock treatment. Psychosom. Med. **14:** 347–362.
16. Alexander, L. 1955. Epinephrine-mecholyl test (Funkenstein test). Its value in determining the recovery potential of patients with mental disease. Arch. Neurol. Psychiat. **73:** 496–514.
17. Moriarty, J. D. 1956. Epinephrine-mecholyl test (Funkenstein test) as a prognostic guide for various physiodynamic therapies. Confinia Neurol. **16:** 105–115.
18. Rouleau, Y. & M. Coulombe. 1957. Clinical applications of the Funkenstein test to psychiatric patients. Can. Med. Assoc. J. **77:** 116–121.

Discussion of the Paper

Theodore R. Robie (*Eastern Psychiatric Research Association, East Orange, N. J.*): Leo Alexander and I have been closely allied for many years in various endeavors directed toward better psychiatric treatment. Concerning iproniazid, however, my own extensive clinical experience leads me to a somewhat different evaluation of the effectiveness of the drug in the treatment of depressions.

It is well known that psychiatric nomenclature is not yet at a level of scientific exactitude comparable to diagnostic classification in other areas of medicine. Appendicitis, mitral stenosis, pernicious anemia, fracture of the tibia—all of these are etiologically distinct entities that can be diagnosed exactly by objective means. The same cannot be said of the psychiatric labels involutional melancholia, manic-depressive depression, or reactive depression or anhedonia, a form of neurotic depression to which Alexander and Berkeley addressed themselves. All of these conditions tend to merge, so much so that it is practically impossible, at a medical staff meeting, to reach 100 per cent agreement on a single diagnosis for a given patient in any psychiatric hospital, despite the observation of the patient by a number of staff psychiatrists for one month or even longer.

However, when suicide occurs it makes little difference by what name the depression was diagnosed. The important point is that suicide was a possible consequence of the depression, whatever its type, and therefore the therapy most likely to have prevented it was indicated. A death from suicide is just as irreversible whether the diagnosis is reactive depression or involutional melancholia, and the danger is equally grave in either category of depression. Therefore, it must be emphasized, as I have done in the past, that iproniazid therapy is indicated in any type of depressive or melancholic illness. To suggest that iproniazid be reserved for anhedonia alone is to assume that this is a clearly distinguishable disease entity, an assumption that is not warranted by clinical experience. It would be impossible to achieve concurrence on such a clear-cut diagnosis in a mentally depressed patient; more important, iproniazid has been shown to be indicated in all retarded depressions, whatever the category. Only in the rare case where it proves ineffective, or if serious complications occur, would there be indication for another, and perhaps less adequate, chemotherapeutic agent.

Alexander and Berkeley have stated that the success with physical treatment during the past 20 years has tended to blur the outlines of the nosological entities that had been painstakingly drawn during the preceding century. In my opinion, this is a typical defense of the descriptive nomenclature of Emil Kraepelin that still clutters up our textbooks in psychiatry. There are many today who see the dawn of a new day for psychiatry, a day when a valid etiological nomenclature will emerge as a result of the discovery that the amine oxidase inhibitors make possible more efficient utilization of the ever-available store of serotonin in the brain. As a consequence, much greater human productivity in motor and intellectual functions is induced.

It is well to point out that not very long ago we were forced to depend upon one of four different kinds of *Pneumococcus* serum to cure pneumonia; if we did not have the right antiserum, the patient often died. Today this is no longer a problem; it has been solved by the advent of penicillin, a specific for all types of pneumonia. What is no longer a useful diagnostic classification was thus ruled out by a later discovery that makes our interest in it merely historical. The situation in psychiatry today is exactly analogous. The four or more types of melancholia, each with a different descriptive term are not diagnostically distinct from a therapeutic point of view.

Our medical problem is vastly simplified by the fact that iproniazid, in the majority of cases, appears to be a specific for depressions of whatever origin and irrespective of the diagnostic category in which we place them. To recall Abraham Meyerson's concept of anhedonia[5] that was considered progressive in the 1920s and to single out this form of depression exclusively for iproniazid therapy is, in my opinion, a step backward. The assertion that iproniazid is useful only for this allegedly "inert" psychasthenic-anhedonic reaction is not borne out by objective evidence adduced here; furthermore, it goes counter to the evidence of many other investigators who have achieved therapeutic success with iproniazid for a variety of depressions: reactive depressions, manic-depressive depressions, involutional melancholia, and various subgroups that we are called upon to treat in our daily practice.

CLINICAL EXPERIENCES WITH AMINE OXIDASE INHIBITORS IN PSYCHIATRY

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The psychotropic effect of a series of hydrazides, all of which are amine oxidase inhibitors, has been under study in 300 hospitalized patients over a period of 2 years.[1-4] The compounds listed in TABLE 1 were evaluated.

Our most extensive experience has been with the first 4 compounds. All of the hydrazides investigated exert an effect of varying intensity upon the basic mood in depressive states. For reasons that cannot yet be definitely answered, clinically iproniazid (Marsilid*), the benzene analogue RO 4-1027, and the serine hydrazide are superior to the other hydrazides. The effect of this first group is characterized by a release from inhibition of psychomotor activity and that of the second group by a decrease in excessive psychomotor activity. The psychotropic effect of the hydrazide group is thus recognized by a modification, first, of the basic drive and, a little later, of the basic mood. Other psychic functions, such as perception, orientation, and intellection are unmodified. This specific action of the hydrazides upon affect and activity, which we have been unable to demonstrate in normal subjects,[3] may be described as clinically significant, individual features of each hydrazide. Five of these features are given below.

(1) The antidepressant effect of the hydrazide group consists in elevating the basic mood toward syntonia. It does not begin suddenly, but develops gradually from one phase to another. This "psychic energizer" (Kline[5]) or "psychoanaleptic" effect (Delay[6]) does not depend on the condition treated, that is, the "depressive syndrome," or on the nature of the mental disorder. As Maurer[7] has shown for iproniazid, the hydrazides in general are effective against the depressive syndrome regardless of its etiology, which may be endogenous-cyclothymic, exogenous-reactive, or a regressive-reactive-neurotic disorder, a psychosis of the schizophrenic type, or a menopausal, involutional, or presenile disorder. The nosological attribute of the depressive syndrome does not determine the efficacy of the hydrazides.

(2) The amine oxidase-inhibiting properties of the hydrazides differ quantitatively. The antidepressant effect of this group of drugs is associated with a definite dosage-time relationship, which is specific for each hydrazide. The more intensively monoamine metabolism is altered by the enzyme-inhibiting process, the shorter the period before the first psychotropic effects are observed. TABLE 2 shows the relationship between dosage and onset of the first psychotropic effects.

Just how differently the monoamine metabolism is influenced by each of the hydrazides is shown by the following results. An estimate of the extent of modification of serotonin metabolism can be obtained from the urinary excretion of 5-hydroxyindolacetic acid (5-HIAA).[8] If the excretion rate of 5-HIAA is maximally increased by pretreatment with a total dose of 750 mg.

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* Hoffmann-La Roche.

of tetrabenazine—a newly synthesized tranquilizer—given over a 5-day period, hydrazine administration on the sixth day decreases the urinary content of 5-HIAA. This decrease is proportionate to the amine oxidase-inhibiting potency of the hydrazide used. Under these experimental conditions, serine hydrazide reduces 5-HIAA excretion within the first 8 hours; iproniazid requires an average of 24 hours, and benzylpicolinylhydrazide 48 hours or more. The difference in the onset of psychotropic action of these hydrazides parallels their effect on monoamine metabolism.

TABLE 1
HYDRAZIDES EVALUATED IN THIS STUDY

First group	*Aromatic acid series*	
	1-Isonicotinyl-2-isopropylhydrazine	Iproniazid (Marsilid)
	1-[p-Chlorobenzoyl]-2-isopropylhydrazine	RO 4-1027
	Amino acid series	
	DL-Serine isopropylhydrazide	RO 4-1038
Second group	L-Glutamic acid α-isopropylhydrazide	RO 4-1385
	Benzyl series	
	1-Benzyl-2-trimethylacetylhydrazine	RO 4-1634
	1-Benzyl-2-picolinylhydrazine	RO 5-0700

TABLE 2
RELATIONSHIP BETWEEN DOSAGE AND ONSET OF FIRST PSYCHOTROPIC EFFECT

Hydrazide amine oxidase inhibitor	Daily dose	Onset of psychotropic effect after
DL-Serine isopropylhydrazide	150 mg.	6 hours
L-Glutamic acid α-Isopropylhydrazide	150 mg.	3 days
	75 mg.	6 days
1-[p-Chlorobenzoyl]-2-isopropylhydrazine	150 mg.	10 days
1-Isonicotinyl-2-isopropylhydrazine	150 mg.	14 days
1-Benzyl-2-trimethyl acetylhydrazine	60 mg.	14 days
1-Benzyl-2-picolinylhydrazine	150 mg.	>25 days

(3) The therapeutic effect of the hydrazides occurs in 2 phases: a more or less marked antidepressant effect, preceded by a subjectively and objectively recognizable modification of drive.

Iproniazid, the benzene analogue RO 4-1027, and the serine hydrazide increase drive. The interval between the first psycho*motor* and the second *psycho*motor phases depends on the particular hydrazide used. With iproniazid the interval is 1 to 2 days, but with serine hydrazide it is often only a few hours. The psychotropic-psychoanaleptic effect of these 3 hydrazides is extremely marked, and their inhibitolytic and antidepressant action is greater than that of the other drugs.

The remaining 3 hydrazides, glutamic acid hydrazide and the 2 benzyl hydrazides, also have this biphasic action, beginning with a reduction of drive and followed by an elevation of mood. It occurs, however, only in patients suffering from psychomotor excitation and agitation with hypochondriacal complaints, tearfulness, or internal agitation and hyperactivity. In depressed and inhibited patients these 3 hydrazides cause dullness, increased fatigue, and lack of initiative; that is, they intensify the syndrome of depressive inhibition of activity. Occasionally they do not modify the psychopathological condition at all, but appear to be completely inert even if administered over a 24-day period. The weakest effect is shown by benzyltrimethylacetylhydrazine. The biphasic action is most clearly seen with benzylpicolinylhydrazine. In depressions associated with increased activity and hypochondriacal fears, this aralkyl hydrazide derivative at first exerts an inhibiting influence on drive, which is later transformed into a lifting of the depressive mood.

Glutamic acid hydrazide is interesting because its psychotropic effect in the depressed inhibited patient depends on the dosage: a daily dose of 75 mg. for about 6 days exerts at first an activating, and later an antidepressant effect, whereas 150 mg. daily produces a diminution of activity.

The psychotropic effect that typifies the hydrazide group is antidepressant. In each case, however, the tendency to normalize drive varies according to the hydrazide used and the initial state of the patient.

(4) The two groups of hydrazides differ not only in their positive or negative influences on drive, but also in their somatic side effects. Those hydrazides that reduce depression cause a reduction of the resting blood pressure that may be associated with disturbed postural circulatory adjustment. There is an increased appetite, which may become ravenous and cause a gain in weight. The extracellular fluid volume (Scheinberg's thiocyanate space[9]) increases rapidly; there is salt retention and loss of serum potassium (K^+) and inorganic serum phosphate, accompanied by reduced urinary excretion of K^+. This intracellular accumulation of K^+ occurs between the initiation of treatment and the appearance of increased psychomotor activity; the electrolyte balance is then restored even if medication is continued. Electropermeagraphic investigations (method of Krause *et al.*[10]) confirm the increased hydration of tissues during the first treatment period. My colleagues and I have observed basically similar electrolyte and fluid alterations in the first phase of therapeutic insulin coma.[11]

Those hydrazides that cause a reduction of excessive drive do not reduce the resting blood pressure. In occasional cases there may even be a temporary increase of previously normal pressure. While body weight increases somewhat, the electrolyte changes are of lesser degree, as is the increase in tissue hydration and extracellular fluid volume. The correlation between the limited increase in hydration and thiocyanate space in the first phase of treatment and the unexpectedly limited decrease in the initially high 5-HIAA content of the urine caused by these hydrazides is noteworthy. A similar correlation is found after a single electroshock treatment.[12] There is as yet no adequate explanation of these results. It can be stated only that a hydrazide induces alteration of membrane permeability with electrolyte imbalance and body fluid retention.

Serial tests of liver function showed no deviations from normal following treatment with iproniazid or other hydrazides.

(5) Reserpine and tetrabenazine on the one hand and psychotropic hydrazides on the other exert opposing influences on monoamine metabolism. Administration of reserpine or tetrabenazine after pretreatment with psychotropic hydrazides, especially iproniazid or serine hydrazide, leads to temporary disturbance of affect and memory[1-3] (lasting up to 3 days). This may be associated with disorientation, illusions of experience and recognition, delirious agitation, and a series of manifestations of autonomic excitation. A similar type of exogenous reaction has been encountered with iminodibenzyl treatment following pretreatment with iproniazid.[2-3] It is therefore advisable after treatment with psychotropic hydrazides to wait at least 3 to 5 days before continuing treatment with reserpine, tetrabenazine, or iminodibenzyl.

I shall add four comments regarding individual hydrazides.

(1) Iproniazid has an established place in the psychopharmacological armamentarium. Doses of 150 to 300 mg. should be given daily until the onset of increased activity, which may be expected within 12 to 14 days of treatment; thereafter 75 to 150 mg. per day should be given for another 2 to 3 weeks. Successful treatment with iproniazid may be expected with a high degree of probability as long as the main depressive symptom is psychomotor inhibition.[3] Other depressive syndromes, for example, those combined with agitation and excitability, may be intensified by iproniazid treatment.

Iproniazid is well tolerated, as are other hydrazides; photosensitivity and allergic phenomena have not been observed, nor was there any indication of liver cell damage in our series of patients. Reduction of blood pressure due to iproniazid is observed, particularly in the first two weeks of treatment, and appears to be related to dosage. Postural circulatory disorders should in no case be treated with drugs containing adrenaline or noradrenaline, which may lead to hypertensive crises, since the hydrazides delay degradation of exogenous catecholamines.

We have treated more than 150 depressed patients with iproniazid. If the duration of treatment up to complete remission is compared with that of chlorpromazine, iminodibenzyl, or electroshock therapy, iproniazid therapy is shorter, the average being only 41 days.

(2) The benzene analogue RO 4-1027 shows little difference from iproniazid in the dosage required, duration of treatment, psychotropic effect, and side effects. The increased activity in depressive inhibition begins usually on the sixth to eighth day of treatment, and the improvement of affect some 2 days later. The latter is in many cases more intense than after iproniazid, while the side effects are approximately the same. Doses exceeding 450 mg. daily cause hypermotility and other phenomena of motor agitation, and constipation.

(3) The effect produced by serine hydrazide in doses not exceeding 400 mg. within 3 days serves as a classic example of the characteristic psychoanaleptic action of the first group of hydrazides. This effect includes a biphasic action after a very brief latent period, and marked improvement of activity and affective state, where a condition of depressive inhibition is predominant. This massive intermittent therapy should be terminated after 3 days to avoid the danger of postural circulatory disorders that might occur by the sixth day. After an interval of 6 to 8 days similar massive therapy of 400 mg. for 3

days can be repeated several times without side effects. As long as these precautionary measures are observed, serine hydrazide is the optimally effective hydrazide in depressed inhibition.

(4) Benzylpicolinylhydrazine is well tolerated, and in our limited experience did not have a marked hypotensive effect. The psychotropic effect in agitated depression may be expected after about 12 to 14 days of treatment. The desired reduction of excessive activity occurs sometimes even earlier. Adequate and lasting therapeutic effects were obtained in 28 of 36 patients with agitated forms of depression after a daily dosage of 60 mg. for about 20 days. In 12 cases of depression with inhibition of activity, this hydrazide was not effective.

To sum up, the psychotropic effect of hydrazides, which parallels their inhibition of amine oxidase, occurs in two phases: it affects first the activity and then the mood. While affect is modified by all hydrazides in an antidepressant direction toward syntonia, activity is either increased or diminished by the different hydrazide derivatives, depending on the initial condition. The therapeutic efficacy of these psychotropic substances is unmistakable, and their clinical usefulness has been demonstrated. The indication for this treatment is the depressive syndrome of varying etiology. The close therapeutic relationship between amine oxidase-inhibiting hydrazides and psychopathological alterations of drive and affect suggests that there is a correlation between basic psychological functions and monoamine metabolism in the brain.

References

1. Voelkel, A. 1958. Klinische Wirkung von Pharmaka mit Einfluss auf den Monoaminstoffwechsel des Gehirns. Confinia Neurol. **18:** 144.
2. Voelkel, A. 1958. Die Therapie der depressiven Hemmung mit dem Monoaminoxydaseblocker "Marsilid." Intern. Meeting Neuro-Psycho-Pharmacol. Rome, Italy. In press.
3. Voelkel, A. 1958. Die Behandlung psychomotorischer Hemmungszustände mit dem Aminoxydasehemmer Marsilid (Iproniazid). Klin. Wochschr. In press.
4. Pletscher, A., F. A. Steiner & A. Voelkel. 1958. Behavioral changes in animals and man caused by drugs which influence monoamine metabolism in the brain. Intern. Meeting Neuro-Psycho-Pharmacol. Rome, Italy. In press.
5. Loomer, H. P., J. C. Saunders & N. S. Kline. 1957. A clinical and pharmacodynamic evaluation of iproniazid as a psychic energizer. Psychiat. Research Repts. **8:** 129.
6. Delay, J. 1958. Discussion. Intern. Meeting Neuro-Psycho-Pharmacol. Rome, Italy.
7. Maurer, H. 1958. Therapeutische Erfahrungen mit Iproniazid (Marsilid "Roche") an einem psychiatrischen Krankengut. Schweiz. med. Wochschr. **88**(25): 617.
8. Udenfriend, S., E. Titus & H. Weissbach. 1955. The identification of 5-hydroxy-3-indoleacetic acid in normal urine and a method for its assay. J. Biol. Chem. **216:** 499.
9. Scheinberg, I. H. & H. J. Kowalski. 1950. The binding of thiocyanate to albumin in normal human serum and defibrinated blood with reference to the determination of "thiocyanate space." J. Clin. Invest. **29:** 475.
10. Krause, R. A., H. Storz & A. Voelkel. 1957. Gleichstrommessungen an der menschlichen Haut bei Quellung und Entquellung des Gewebes. Klin. Wochschr. **35:** 674.
11. Voelkel, A. 1956. Beitrag zum Kaliumstoffwechsel im therapeutischen Insulinschock. Arch. intern. pharmacodynamie. **108**(2): 135.
12. Voelkel, A. 1957. Elektrokrampf und Monoaminstoffwechsel des Gehirns. Naturwiss. **44**(20): 541.

Discussion of the Paper

Henry A. Peters (*University of Wisconsin College of Medicine, Madison, Wis.*): If Emil Kraepelin were living today, he would be extremely interested and pleased with this publication. In the light of his belief in the organic causation of the major psychoses, he might well have predicted the subject under current discussion.

Voelkel's group of more than 350 patients is impressive; more than one half of them were suffering from various acute types of depressive reaction. In our own series of 30 patients, 17 of whom revealed psychotic depressive symptoms, we arrived at similar conclusions regarding the effectiveness of iproniazid in psychotic depression, although we did not have experience with some of the new hydrazides described by Voelkel. Fortunately, such symptoms as weight loss, early-morning awakening, and ideas of self-deprecation are objective signs that can be utilized to determine the effectiveness of any treatment for psychotic depressive reactions. In our group of 17 patients, 8 had relapsed following electroshock treatment; subsequently all of them responded to iproniazid therapy. Three patients undoubtedly would have become chronically institutionalized were it not for the effectiveness of iproniazid therapy, because repeated courses of electroshock treatment alone had failed to produce a remission. One of the most interesting aspects of iproniazid utilization is its tendency to worsen, at times markedly, the symptoms of acute or latent schizophrenia, increasing all the secondary symptoms of the disorder, as described by Bleuler.[1]

There are certain neurological diseases that seem to be direct opposites of each other: for example, myasthenia gravis as opposed to myotonia. While myasthenia gravis is helped by medications such as neostigmine (Prostigmine*) and ephedrine, myotonia may be aggravated by these same drugs. Parkinson's syndrome seems to represent the antithesis of the choreic disorder; if one administers high doses of reserpine (Serpasil†) to the choreic, one induces Parkinson's syndrome, which virtually eliminates the choreic features of the disorder.

We may think of the schizophrenic patient as a direct opposite of the depressive patient. The schizophrenic tends to worsen clinically, following the administration of adrenaline, amphetamines, or Marsilid, all drugs used in the treatment of depression. On the other hand, drugs such as chlorpromazine and the *Rauwolfia* compounds, which are useful in controlling schizophrenia, often may precipitate or aggravate depressive illness.

In our patient group we were struck by the difference in age between the schizophrenics, who averaged 29 years of age, and the depressive patients, who averaged 59 years of age. With advancing age, could change in the blood-brain barrier account for the different response to Marsilid administration? We further note that the schizophrenics tend toward hypotension, whereas the depressive patients are often hypertensive. Might the schizophrenic metabolism deal differently with the catecholamines to produce hallucinatory and other symptoms while, in the depressive patient, these metabolic materials produce mainly vasopressive substances and hypertension? Voelkel has pointed out that reserpine or tetrabenazine administered to patients pretreated with the psychotropic hydrazides have an explosive effect on both affect and memory. One can imagine the psychiatrist treating the depressive aspects of involutional psychosis with iproniazid, following which the internist administers reserpine in order to reduce the blood pressure. Clearly, in this age of psychiatric medical therapy, the psychiatrist must become more of a clinician and vice versa. The potential danger of this sequence of drug administration is not generally appreciated, and those working with these agents should be alerted to it.

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* Hoffmann-La Roche.
† Ciba.

Voelkel brought up an interesting point showing how, with the administration of serine hydrazide, he must pause after the first 3 days of medication for an interval of 8 or more days before resuming treatment to avoid inducing a hypotensive crisis. One wonders just what adaptive change occurs during this interval to increase the patient's tolerance to the hypotensive effect of this drug. The high dosages of iproniazid (300 mg.) and serine hydrazide (400 mg.) reported by Voelkel seem heroic, although it is reassuring to note that his patients tolerated these levels during his shock therapy. Generally, we utilized much lower dosage levels in our patient group.

The comments concerning glutamic acid hydrazide were also of great interest. Voelkel noted that daily doses of 75 mg. of this drug removed symptoms of depression and inhibition but that, in doses of 150 mg. per day, inhibition is unaccompanied by any analeptic effect. One wonders whether this represents a hyperserotonin effect, perhaps analogous to the hypercholinergic response in myasthenia gravis when an excess of Prostigmine has been administered.

The studies in which increased hydration and intracellular potassium are restored to normalcy during the initial stages of hydrazide therapy are important, and they likewise call attention to such entities as myasthenia gravis, narcolepsy, and familial paralysis in which the potassium ion and hydration seem to play an important part. Studies with radioactive potassium might be of additional help.

RO 5-0700, which seemed capable of calming the agitative depressive patient in Voelkel's series without altering urinary 5-hydroxyindole acetic acid excretion, is noted. This medication may be of value in the schizoaffective or manic group of patients.

Finally, I call attention to the work of Winifred Ashby in determining the carbonic anhydrase content of the central nervous system and its alteration in the brain of the schizophrenic and the general paretic, and in other organic psychoses.[2-4] When an electric current is passed through the hypothalamus, the carbonic anhydrase levels decreased significantly. What is the effect of Marsilid on this enzyme system?

References

1. Bleuler, E. 1950. Dementia Praecox, or the Group of Schizophrenias. J. Zinkin, Tr. Intern. Univs. Press. New York, N. Y.
2. Ashby, W. 1947. The pattern of enzyme distribution in the brain and mental function. J. Nervous Mental Disease. **105:** 107.
3. Ashby, W. & G. D. Weickhardt. 1947. Distribution of carbonic anhydrase in the pallium in nineteen cases of paretic neurosyphilis. J. Nervous Mental Disease. **106:** 107.
4. Ashby, W., M. Bushrod & E. M. Schuster. 1957. Procedures changing the carbonic anhydrase activity of the central nervous system. Diseases of Nervous System. **18:** 226.

CLINICAL EXPERIENCE WITH NEWER ANTIDEPRESSANT DRUGS AND SOME RELATED ELECTROENCEPHALOGRAPHIC OBSERVATIONS

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Investigative work to determine the effect of drugs on psychotic processes has a long history at Longview State Hospital beginning with the treatment of neurosyphilis in 1926 and the use of insulin, pentamethylene tetrazol (Metrazol*) and electroshock in the 1930s. The trial of drugs for treatment of psychoses began during the 1940s and has become the principal treatment activity of the hospital since the introduction of chlorpromazine and reserpine in 1954. It was evident in the first year's experience that phenothiazines and reserpine were useful chiefly in schizophrenia and the manic phase of manic-depressive psychoses. Depressions were relieved only occasionally, and were precipitated with troublesome frequency during treatment with phenothiazines or reserpine in previously nondepressive individuals, or were activated in patients in whom depression had been dormant or had subsided. Until the studies of iproniazid (Marsilid†) clarified its field of usefulness, only electroshock and its variants were available for treatment of depression.

The early reports of Kline and his co-workers[1] did not indicate a specific group in which iproniazid was useful except that inert, inactive, withdrawn, and depressed individuals seemed to benefit. Careful study of their case reports was not encouraging. After about 50 of our patients under treatment with iproniazid had been observed over a period of several months, the specificity of the beneficial effect of iproniazid on depression gradually became evident. Also evident was its action to precipitate manic states in cyclic manic-depressive illness and to intensify overt schizophrenic psychoses or arouse latent ones. This specific and differentiating effect of iproniazid seemed sufficiently interesting to justify further work at a point where interest in this substance had otherwise declined.

With this orientation to the use of the drug, further work became clearly gratifying and served as a background for investigation of more recently proposed compounds for the treatment of depressive reactions. It was natural in our hospital that electroencephalograph (EEG) studies should be carried out for comparison with the EEG studies in schizophrenic psychoses. We believe that correlation of clinical and EEG studies is leading to a new understanding of the clinical and physiological differentiation of psychotic states.

For the purposes of this report, depression must be clearly defined. Many illnesses—not only psychiatric maladies—have aspects that may be considered depressive. The state of discouragement associated with many chronic diseases (orthopedic, neurological, and malignant) may justifiably be considered as depressive reaction, as may also the normal grief of bereavement. However, these are relatively appropriate reactions. Similarly, lethargy associated with physical illness such as narcolepsy, hypothyroidism, diabetes, and myasthenia can be considered depressive whether the origin of the sluggishness is physical, emotional, or both. Nevertheless, none of these or similar conditions can be considered fundamentally similar to the totally inappropriate state of psychotic depression that has been described at times as dysphoric or endogenous and is typified in the depressive phase of manic-depressive psychosis. Depression of this variety cannot be relieved by pharmacological stimulation or endocrine substitution; such treatment may even accentuate the depression. These depressive states are characterized by feelings of sadness, weeping, ideas of unworthiness and inferiority, anxiety, guilt, and feelings of having done wrong, with or without the attempt to rationalize the feeling to environmental factors; ideas of delusional proportion regarding malfunction of one or more organ systems and, at times, actual dysfunctions that must be considered components or effects of emotional states and as the typical masquerades of depression; anger in overt or suppressed form; and, probably of most specific importance, suicidal drive. These depressive states are compounded of physiological and psychological parts, none of which is a proper and suitable adaptation to any immediate environmental stimulus, yet the individual involved remains oriented and with clear sensorium and perceptive ability. Schizophrenic suicidal drives, which may occur under the pressure of fear generated by bizarre and terrifying hallucinations, must be distinguished clinically, chiefly by the difference in sensorium and perception.

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* Bilhuber-Knoll.
† Hoffmann-La Roche.

It is such psychotic depression that is specifically benefited by iproniazid and some newer antidepressant compounds. That this is a specific effect may be deduced from reference to a previous study carried out in 1953 in which pipradrol hydrochloride (Meratran*) was administered to 120 psychotic patients and, simultaneously, amphetamine was given to 60 other psychotic individuals as contrast for a period of 3 months. Neither of these potent stimulants relieved depression or aggravated manic or schizophrenic states in these large groups of patients. However, in the present study of the effect of antidepressant drugs such depressive reactions were resolved, and the patients maintained in what may be called a euthymic state in a manner not previously observed in many years of experience, even with the use of Metrazol and electroshock.

In anticipation of what must be considered anachronistic skepticism, it is immediately stated that the drugs were administered under conditions that make spurious results highly unlikely. At Longview Hospital a sophisticated team of nurses and specially chosen psychiatric technicians, under the guidance of experienced physicians headed by myself, administer the drug-treatment program. At any one time more than 2000 inpatients and more than 500 outpatients receive drugs for relief of psychotic states. Regular observations are made and recorded, and any unusual occurrence is immediately referred to the director or his deputy. Skepticism is cultivated, and "yes men" are dropped from the team. After 4½ years and the treatment of 5000 patients, double-blind studies with adequately effective compounds would be futile.

Drugs were administered orally except to those patients who refused or objected; these received intramuscular medication. The usual routine was medication 3 times daily but, after an adequate effect was achieved, once daily was often sufficient. In the early studies with iproniazid, 50 mg. 3 times daily was the beginning dose, but after a number of patients developed severe orthostatic hypotension, the starting dose was reduced to 25 mg. twice daily except in hypertensive individuals, to whom 25 mg. 3 times daily was given. Phenethylhydrazine (W-1544) was given in doses of 25 mg. 3 times daily, but it was soon evident that less was usually better tolerated, and medication twice daily was more common. The dosages of other drugs are indicated in TABLE 1. After adequate recovery the dose of iproniazid for maintenance can often be reduced to 5 mg. daily.

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* Merrell.

Relief of depressive manifestations does not occur with predictable speed, but in most patients it begins in 7 to 10 days and then progresses gradually to full effect over the next 3 to 6 weeks. Occasional previously untreated patients respond within 2 or 3 days with brightened affect and renewed extroverted interest, and some, after long chronic illness resistant to all previous measures, only begin to show signs of favorable effect after 4 weeks and require 3 months for full benefit. Occasional individuals have a period of increased symptoms, chiefly agitation, before improvement occurs. It is important not to be influenced by such reactions in the first 2 weeks of treatment. This is particularly true of iproniazid; other drugs have not been under observation for a period of sufficient duration to allow comparison in this respect. After relief is well established, careful observation must be continued to regulate dosage, usually downward, and to recognize late unfavorable effects.

TABLE 1
DOSAGES

	Single dose, mg.		Daily frequency
	Minimum	Maximum	
Iproniazid (Marsilid)	5	50	once or twice
G-22355 (Imipramine)	25	100	twice to 3 times
W-1544 (phenethylhydrazine)	12.5	50	once or twice
MKDP	25	100	3 times
SKF 385	10	50	2 to 3 times

The first clearly recognizable useful effect of iproniazid occurred in a private patient who had become unsuitable for electroshock treatment that had relieved a depressive reaction 2 years previously. He was obese, had a variable cardiac arrhythmia, and did not respond to treatment with phenothiazines. Within 1 week after iproniazid was started, he appeared cheerful, interested in his farm and business interests, and had renewed confidence in himself and his family. His response was in every way analogous to that of the deluded and hallucinating schizophrenic patient who, after a good phenothiazine takes effect, announces that the Communists are not really infiltrating the medical and nursing staff of the very Catholic hospital, and he does not really know how he acquired such a crazy idea. Improvement of this first patient from iproniazid led to its specific use for depressive manifestations in both private and state-hospital patients.

At the time the study of iproniazid was initiated, a large group of withdrawn, inert patients was available in the state hospital and in the private practice.

Table 2
Criteria for Improvement

A: Patients who have recovered from all active psychotic manifestations, particularly those having lost all delusions and hallucinations and having insight into previous delusions and hallucinations, with adequate affect, social and industrial adaptive capacity, equal at least to the premorbid level; and no overt hostility, particularly to the hospital or its staff.

B: Individuals who have recovered from most psychotic manifestations, but who may not have lost belief in previously present delusional experience, who are able to make a satisfactory social and industrial adaptation away from the hospital or in the hospital under privileged conditions, and whose improvement may be summarized as "a good social remission."

C: Patients who have improved from the initial condition in significant ways, particularly in ability to communicate verbally, in diminution or absence of assaultiveness, incontinence, denudativeness, and other gross manifestations of psychosis, and in some diminution of psychopathological manifestations such as delusions and hallucinations without actual resolution of these.

D: Patients who have improved only slightly or not at all.

E: Patients made more psychotic (schizophrenic or manic).

Table 3
Results with Iproniazid in State-Hospital Patients

	A	B	C	D	E
Schizophrenia					
with other drugs					
(*A*) Treated 1 to 3 months	0	0	6	0	1
(*B*) Treated over 3 months	0	5	14	1	3
without other drugs					
(*A*) Treated 1 to 3 months	0	0	2	0	3
(*B*) Treated over 3 months	2	1	5	3	4
Total	2	6	27	4	11
Affective psychoses					
with other drugs					
(*A*) Treated 1 to 3 months	1	1	0	1	0
(*B*) Treated over 3 months	3	2	9	0	1
without other drugs					
(*A*) Treated 1 to 3 months	0	0	1	2	2
(*B*) Treated over 3 months	4	4	8	1	1
Total	8	7	18	4	4

Most of these were already being treated, however inadequately, with various phenothiazine derivatives, and had been for some time. Rather than produce change from drug withdrawal in relatively stable patients, the phenothiazines were continued and the iproniazid added. In the state-hospital result tables the patients are therefore divided into groups treated with and without other drugs in the iproniazid series (TABLE 2).

TABLES 3 and 4 indicate the results obtained from the use of iproniazid in state-hospital and private patients. The preponderance of good results in clearly depressive reactions is immediately evident. The apparently inconsistent *A* and *B* results in schizophrenic state-hospital patients required further investigation; the significant findings are tabulated in TABLE 5. The irregularities, contradictions, inadequacies, and inconsistencies in the diagnosis of schizophrenia in even a well-regulated state hospital are immediately brought to mind. The only patients graded *A* were in the schizoaffective group, and the *B* patients were all among those pretreated unsuccessfully with a phenothiazine compound and further treated with iproniazid. These patients, who were probably improperly labeled as schizophrenics, will presently become a subject of major interest.

TABLE 4
RESULTS WITH IPRONIAZID IN PRIVATE PATIENTS*

	A	B	C	D	E
Schizophrenic patients	0	0	0	2	3
Affective psychoses	52	7	3	0	0

* All treated more than 3 months.

TABLE 5
GOOD RESULTS IN STATE-HOSPITAL PATIENTS CLASSIFIED AS SCHIZOPHRENIC

Patient No.	Diagnosis	Other drug used with iproniazid	Remarks
12	22.01	0	Chiefly depressed, no hallucinations
27	22.51	Triflupromazine	Chiefly depressed, no hallucinations
38	22.51	Triflupromazine	Chiefly depressed, no hallucinations
45	22.31	Perphenazine	Chiefly depressed, no hallucinations
55	22.91	Win. 13,645–5	Withdrawn, depressed
64	22.61	0	Typical depressive of long standing
74	22.61	0	Typical depressive of long standing
123	22.31	Stelazine	Chiefly neurodermatitis

22.01—Simple type
22.31—Paranoid type
22.51—Chronic undifferentiated type
22.61—Schizoaffective type
22.91—"Other" type

The private patients had been known to me for from 1 to 20 years. Of 68 patients considered suitable to grade for improvement of psychotic illness, 55 had been hospitalized 1 or more times, and 53 had had electroshock treatment. Of the latter, only 3 had had a brief period of concurrent electroshock treatment, and all the depressive reactions were subsequently able to do well without electroshock treatment for maintenance and, to date, have had no relapses. Many of the private patients were being maintained in a partial state of improvement by the administration of phenothiazine when treatment with iproniazid was first used, therefore, production of withdrawal symptoms due to discontinuance of the phenothiazine drugs was not risked when iproniazid was started. The differential effect of iproniazid was easily recognizable and, in this group of familiar patients, the quality of the result from drug treatment was far better than any result previously achieved by electroshock treatment. As a result, very little electroshock is now administered in the state hospital, and only occasional private patients require it for the acceleration of drug effect or for the elimination of suicidal risk.

Certain individual private patients also proved noteworthy in illustrating emerging principles. One manic and two schizophrenic patients showed marked exacerbation of symptoms. Several manic-depressive patients were maintained in the *A* group by the addition of phenothiazine or the increase in dose of phenothiazine to abort a threatening manic reaction during iproniazid treatment. Several long-familiar chronic relapsing patients who formerly had required maintenance electroshock or had had one or more hospital admissions per year over an extended period have now been maintained for more than one year with combined phenothiazine and iproniazid treatment in good functioning condition, a state that had not been previously possible with phenothiazines or electroshock alone. Some patients whose advancing arteriosclerotic heart disease makes electroshock more hazardous now need not face this unpleasantness. In some new patients, when time is important, we have not hesitated to add electroshock to the regime for 1 or 2 weeks (4 or 5 treatments) to accelerate discharge from the hospital.

It has been possible to offer such help to some of the state-hospital patients, but the proportion of depressive reactions reaching state hospitals is now much smaller than in the earlier years of electroshock treatment. It may be considered that those reaching the state hospital are a hard core of resistant patients who would not be expected to benefit from electroshock treatment. Nevertheless, our *A* and *B* rate is better than that achieved by electroshock treatment even in this group.

The recognition of occasional rare depressive reactions in well-controlled formerly schizophrenic patients in whom depression is not to be attributed to drug effect is of clinical interest; this is analogous to simultaneous occurrence of 2 kinds of physical illness. One of several such patients is worthy of mention. A woman of 36 who had been followed through and after a severe schizophrenic reaction for 5 years, who had been well and managing her family for at least 2 years, reported one day depressed and tearful. Life hardly seemed worthwhile. Any patient less well known would have been hospitalized and treated with electroshock immediately. This patient, however, had a reasonable precipitating cause. Her youngest child had just entered school and the home had become oppressively quiet. The patient described herself as feeling "homesick." Within 1 week after adding iproniazid to her regime she became cheerful and tolerant of the world's normal stresses.

Other antidepressant drugs have not been under investigation for sufficient time or have not been observed with a sufficiently large group of patients to be compared adequately with iproniazid. Early results with other such drugs are very similar to those seen with iproniazid. Certain similarities in side effects and some results justify further intensive study. TABLE 6 gives the crude data of these studies.

Side effects of iproniazid have been given adequate publicity in the lay press, but insufficient careful medical scrutiny. In the treatment of more than 250 patients, some of whom have been taking iproniazid for over one year, no patients have had jaundice. Orthostatic hypotension, however, is a much more frequent and, when neglected, is potentially a more dangerous side effect of iproniazid. This effect is apparently characteristic of compounds with appreciable inhibiting effect on amine oxidase. TABLE 7 shows blood pressure observations with some of the compounds studied.

The clinical importance of orthostatic hypotension cannot be exaggerated. It is a limiting factor in the use of iproniazid and produces frightening symptoms, but it can be managed if recognized early and if appropiate measures are taken. If neglected, irreversible changes may occur and lead to arterial thromboses in critical organs, hypoxia in brain, heart, or other viscera, with eventually fatal outcome. All patients receiving iproniazid or its analogues should have frequent blood pressure determinations, at least once daily when in the hospital, and twice weekly or more when treated as outpatients until adequate time to observe response has passed. Patients with initial hypertension only rarely become hypotensive and may be given larger initial doses. Hypotensive individuals with blood pressure of 110 or less systolic should not usually be started on these drugs as outpatients. Normotensive individuals achieve adequate benefit from 25 mg. iproniazid twice daily as readily as from

TABLE 6
RESULTS WITH OTHER DRUGS

Drug	A	B	C	D	E
Phenethylhydrazine (W-1544)	0	0	5	1	1*
SKF-385	0	0	6	1	2†
Imipramine (G-22355)	0	1	9	2	3‡
MKDP	0	2	12	2	4§

* Two patients "more psychotic," treated less than 1 month.
† One patient "more psychotic," treated less than 1 month.
‡ Three patients "more psychotic," treated less than 1 month.
§ One patient "more psychotic," treated less than 1 month.

TABLE 7
BLOOD PRESSURE EFFECTS*

	Iproniazid	Phenethylhydrazine (W-1544)	SKF-385	G-22355	MKDP
No. of patients recorded	96	10	8	19	18
No. of patients with drop of blood pressure 20 mm. or more	60	7	5†	3	8
No. of patients with blood pressure below 90 mm. systolic	26	2	2	0	0

* Iproniazid, phenethylhydrazine, and SKF-385 are monoamine oxidase inhibitors; the other two drugs are not.
† Two of these are patients whose blood pressure dropped from hypertensive to normotensive levels.

larger doses. If the blood pressure drops more than 20 mm. Hg or below 100 systolic, the drug should be withdrawn, except in rare special individuals well known to the physician, until the blood pressure returns to safer levels. Determination in upright and recumbent positions must often be made. Fainting during hypotensive reactions may lead to serious physical injuries.

To relieve hypotension, the most effective measure is the administration of cortisone or hydrocortisone in small doses: 12.5 mg. 1 to 3 times daily, or ACTH in slowly absorbed forms (gel or zinc) 20 to 40 units daily for 2 to 5 days, and then continued at lower dosage controlled by blood pressure. If a patient is uncooperative and unreliable in following instructions, it is best to stop the medication until hospital-type control is possible.

Another annoying but not hazardous effect of iproniazid occasionally observed is edema of the extremities. For some unknown reason this may be unilateral. The swelling subsides on reduction in dosage of drug and usually upon addition of large doses of vitamin B complex. Some observations indicate the possibility that pyridoxine (vitamin B_6) deficiency is specifically involved. The use of diuretics is not satisfactory.

Wakefulness and excitement are frequent effects of iproniazid and phenethylhydrazine. Reduction of dosage is the simplest way to modify this effect, but a more specific remedy was discovered as the result of mandatory improvisation for a patient who reacted to phenethylhydrazine with dramatic excitement after 2 days of 25 mg. twice daily, intramuscularly. This patient could not lie down, sit, or stand, his extremities were in constant motion, he could not cooperate, and was resistant to any attempt to feed him. Blood pressure was normal. Twenty-five mg. of triflupromazine intramuscularly every 4 hours for 3 doses and then twice daily, for another day entirely relieved the excited state. Other patients who were overstimulated by these drugs have since been relieved promptly by effective phenothiazine medication even though the stimulation was less intense. Barbiturates are likely to produce some excitement under such circumstances and are not recommended.

Visceral atony is frequently seen in patients receiving iproniazid. This takes the form of stubborn constipation and difficulty of urination. Appropriate measures must be taken to relieve these effects. In the present group of patients the use of large doses of vitamin B complex seemed to have some preventive value. Changes in libido and potency are usually transient if no gross hypotension develops. One woman developed slight lactation, with no other cause apparent.

At least brief mention should be made of the clinical use in nonpsychiatric patients of iproniazid purposely for production of these side effects. Details will be described elsewhere. Patients with hypertension of severe degree required much smaller doses of other antihypertensive drugs or none at all for maintenance of safer blood-pressure levels. In one patient who had required 800 mg. of hydralizine daily to maintain a level of 150 mm. systolic, this level was maintained with only 100 mg. of hydralizine with 150 mg. of iproniazid. Another patient was relieved of enuresis, and several others of manifestations of colitis by the administration of iproniazid. In these patients it is entirely likely that the benefit is derived from the combination of benefit to emotions and physiological processes.

In the study of this relatively large group of patients it is evident that an important clinical correlate of antidepressant activity is the capacity to trigger manic reactions in susceptible individuals and to arouse or intensify schizophrenic psychotic symptoms. This observation is in clear contradistinction to the effect of reserpine and phenothiazines, which can produce depressive reactions in susceptible individuals most frequently in manic-depressive patients as the manic phase is relieved, but also in others less predictably susceptible. This suggests that two adversive groups of illness can be distinguished clinically, and equivalent groups of drugs pharmacologically, by their effects in such illnesses.

It is in relationship to the differentiation of illnesses and drug effects that some EEG observations we have been making during the last three years are of particular interest. Numerous studies of the EEG in psychotic states, made under routine resting conditions and with photic or Metrazol activation, have brought to light no characteristic activity attributable to any psychotic state. With a newer activating technique, however, we believe that specific activity related to psychotic functioning can be demonstrated.

The activation is carried out as follows: during routine EEG recording, Pentothal* is injected intravenously in 2 or 3 doses of 100 mg. at intervals of 2 min. Recording is continued through the period of drowsiness or sleep that follows the injections to obtain a good sleep record in addition to the "activation" record. It is emphasized that neither anesthesia nor sleep is involved, but both are specifically avoided in the period of activation within 2 to 4 min. after the injection.

Characteristics that distinguish "schizophrenic" from other records are: (1) prolonged beta activity of unduly high voltage above a minimum of 25 μv., (2) presence of bursts of fast activity, asymmetrically disposed, present more than 1 min. after injection of Pentothal, and (3) theta activity over 100 μv. amplitude, hypersynchronous for more than 1 min. in more than one lead. Further details will be published later.

The effect of Pentothal by this technique is shown in FIGURE 1. The much weaker effect of Amytal Sodium† is shown for contrast. FIGURE 2 shows a characteristic schizophrenic EEG tracing with details of the specific characteristics, and shows for contrast the EEG from the same individual after treatment with a phenothiazine drug for a period sufficiently long to produce relief from the symptoms of illness. The specific activity is entirely suppressed after recovery from illness. These studies have been carried out in several hundred schizophrenic patients and in a larger number of controls of various kinds. Depressive patients show no specific effect with Pentothal.

Some schizophrenic patients, ill for a long time, do not show the change characteristic of Pentothal activation. Such patients are clinically inert and show minimal active psychotic symptoms. With administration of iproniazid and other antidepressant drugs, however, either intravenously in acute studies or orally over a longer period, the response to Pentothal in the "psychotic" pattern is reestablished.

FIGURE 3 shows a series of EEG tracings in a chronic schizophrenic patient

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* Abbott.

† Lilly.

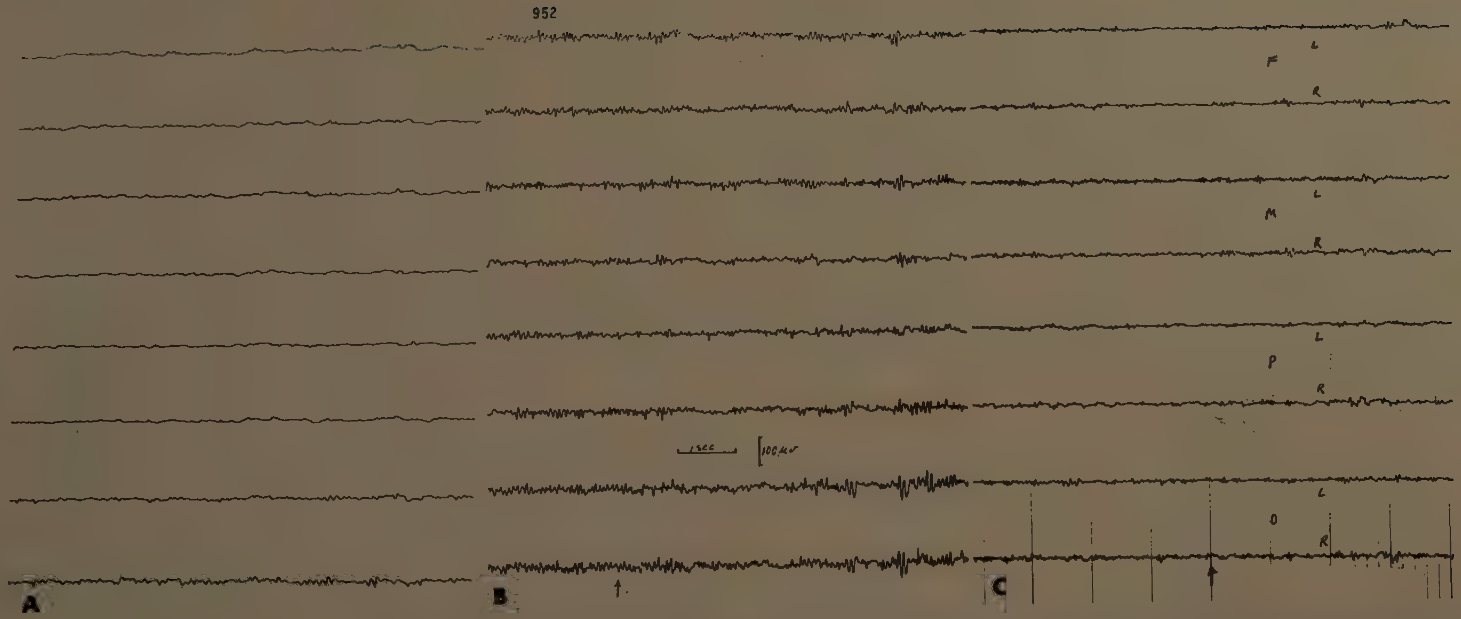

FIGURE 1. Normal male, age 22, hospital attendant. (*A*) Normal resting tracing. (*B*) Tracing after third injection of 100 mg. Pentothal (300 mg. total). Arrow indicates 130 sec. after last injection. (*C*) Similar to *B*, but after third injection of 100 mg. Amytal (300 mg. total). This indicates the response to Pentothal activation in the normal individual for contrast with the following tracings. Time relations are of maximum importance. The reaction within the first 30 sec. may be much greater, but does not persist in the normal individual. Sodium Amytal, a much "weaker" agent, is not suitable for this test.

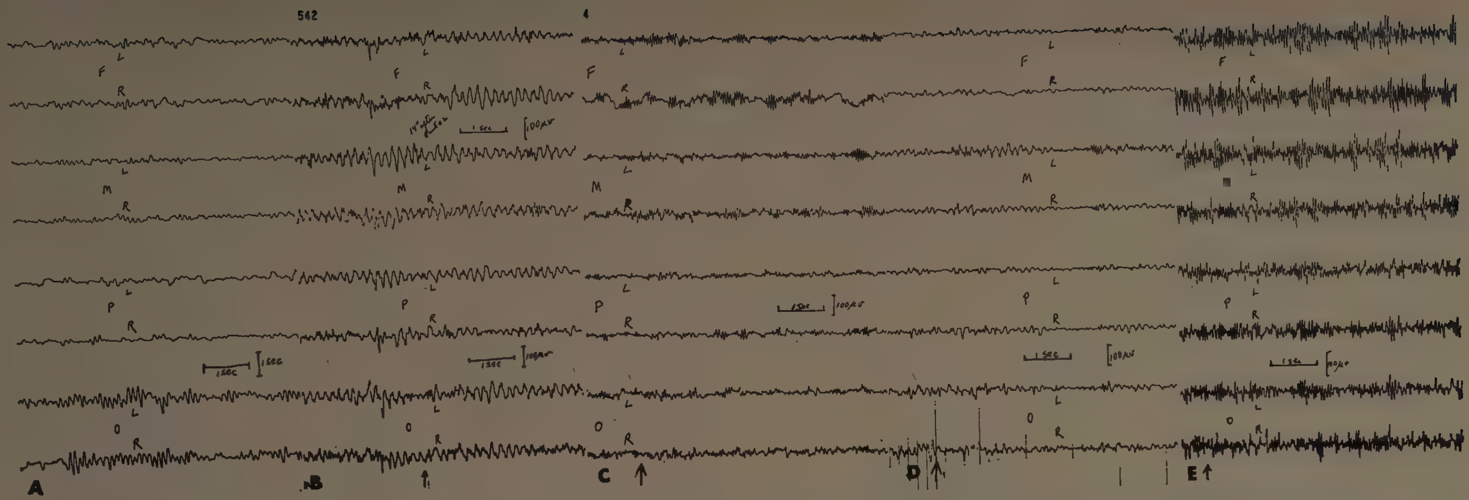

FIGURE 2. Young schizophrenic patient (*A*, *B*, and *C*, before treatment; *D* and *E* after treatment). (*A*) Normal resting tracing. (*B*) Tracing 14 sec. after the second injection of 75 mg. Pentothal (125 mg. total). This section shows theta activity hypersynchronous in more than 1 lead, which is frequently present in "schizophrenic" tracings. (*C*) Tracing after third injection of 75 mg. Pentothal (200 mg. total). This shows the persistence of beta activity and the presence of "bursts," which are asymmetrical and most marked in the more anterior leads. (*D*) Tracing made after patient had been receiving Chlorpromazine (150 mg. 3 times daily) for 3 months. This tracing was made after the second injection of 75 mg. Pentothal (150 mg. total), 120 sec. after the injection. Note the practical absence of the characteristics evident in C above. (*E*) Patient had been out of the hospital for about 6 months, had ceased to take medication, and had returned in complete relapse, with severe psychotic manifestations. This tracing was made after the second injection of 100 mg. Pentothal (200 mg. total—patient had grown and gained weight in the interval). Arrow indicates 72 sec. after injection. There is marked recurrence of the schizophrenic type of activated activity.

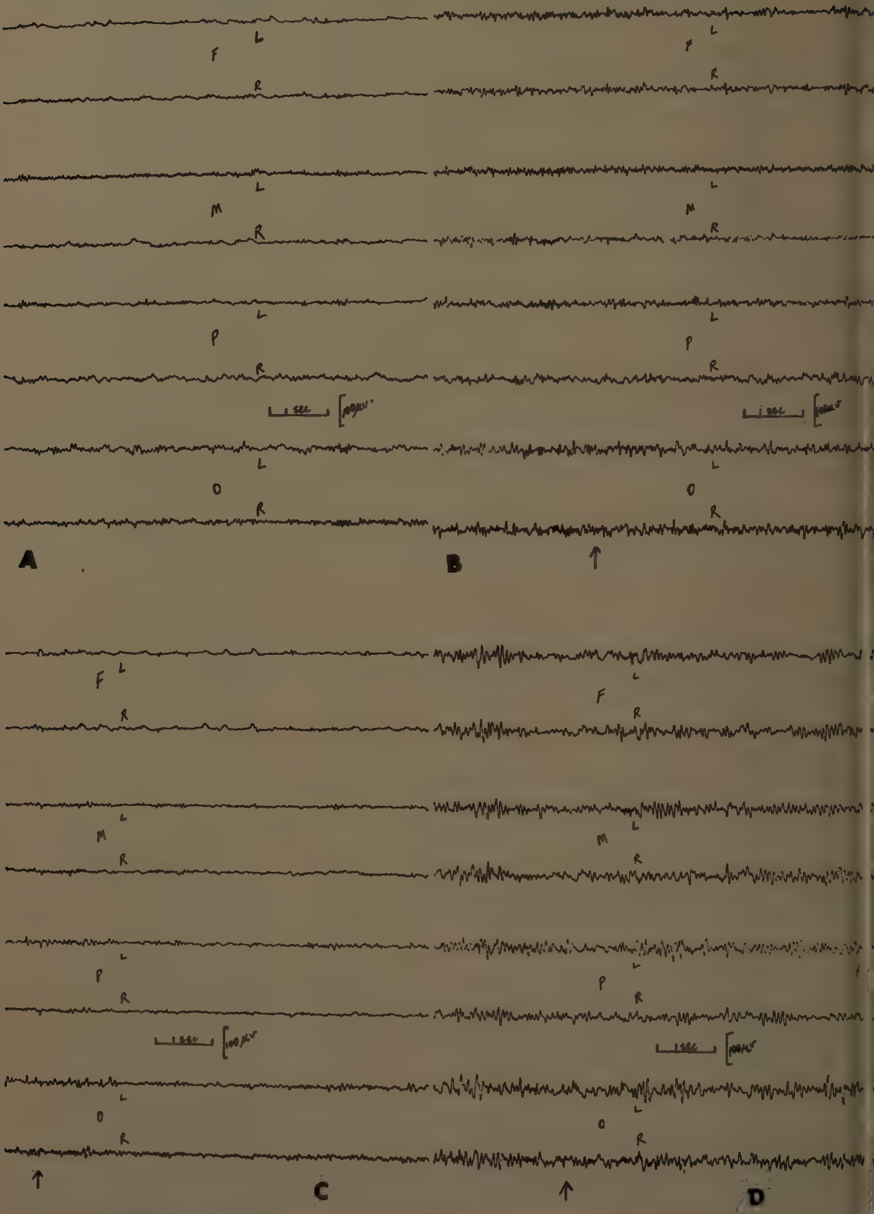

FIGURE 3. Schizophrenic patient admitted to state hospital in 1942. (*A*) Resting tracing prior to treatment. (*B*) Minimal effect of third injection of 100 mg. Pentothal (300 mg. total). (*C*) Tracing after 100 mg. iproniazid 70 sec. after the second injection of 50 mg. (arrow). This portion of the tracing is indistinguishable from the previous record in the normal resting state. (*D*) Tracing shows marked increase in fast activity to significant levels for schizophrenic functioning. Arrow indicates 60 sec. after the third injection of 100 mg. Pentothal (300 mg. total). This is the same record as in *C* above, indicating the effect of intravenous iproniazid.

hospitalized for many years. Initially, Pentothal shows no effect. After intravenous injection of iproniazid, which itself produces no EEG change, Pentothal injection produces the characteristic effect. FIGURE 4 shows a similar series of tracings indicating the effect of oral medication over a two-week

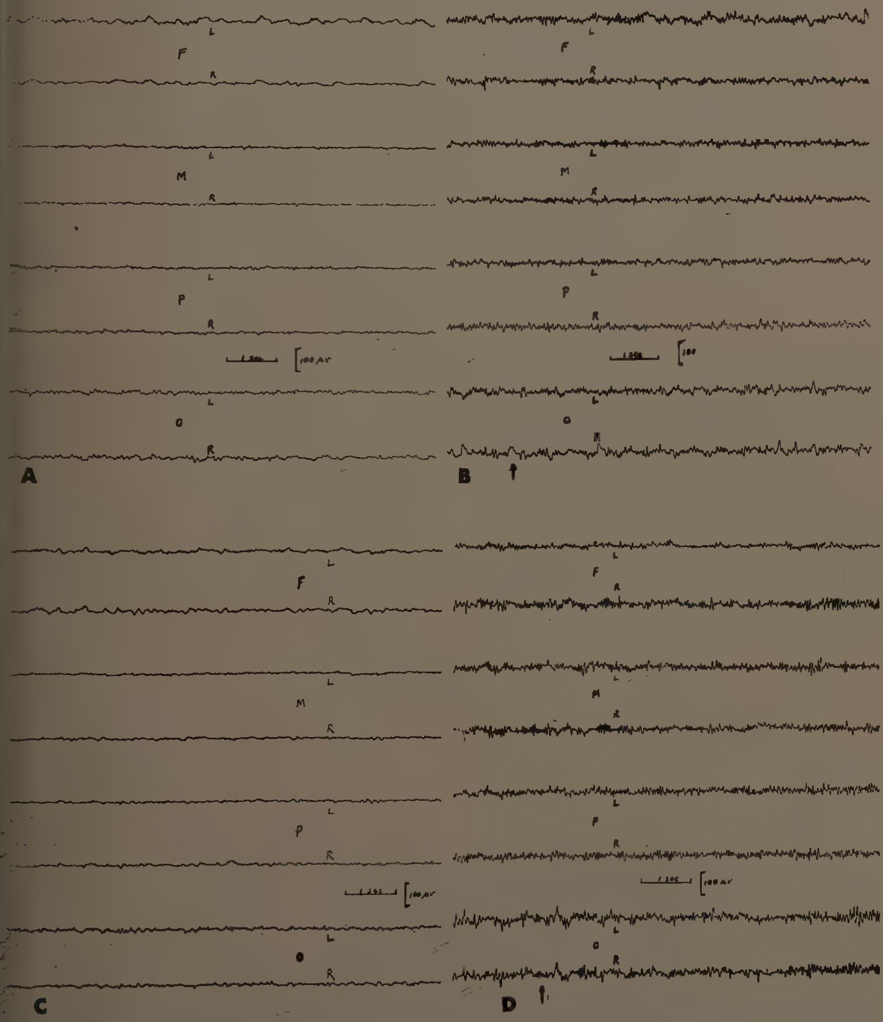

FIGURE 4. Schizophrenic patient. (*A*) Normal resting record prior to treatment. (*B*) Following injection of Pentothal; arrow indicates 100 sec. after the second injection of 100 mg. (200 mg. total). This portion of the tracing shows the minimal Pentothal effect, probably within normal limits. (*C*) Resting normal record after patient has been receiving phenethylhydrazine (W-1544) for 4 weeks. This portion of the record is indistinguishable from the previous record in *A* above. (*D*) Record after injection of Pentothal. Arrow indicates 100 sec. after second injection of 100 mg. Pentothal (200 mg. total). There is a marked increase in fast activity to significant amplitude symmetrical "bursts." This is the same record as in *C* above, indicating the effect of oral phenethylhydrazine.

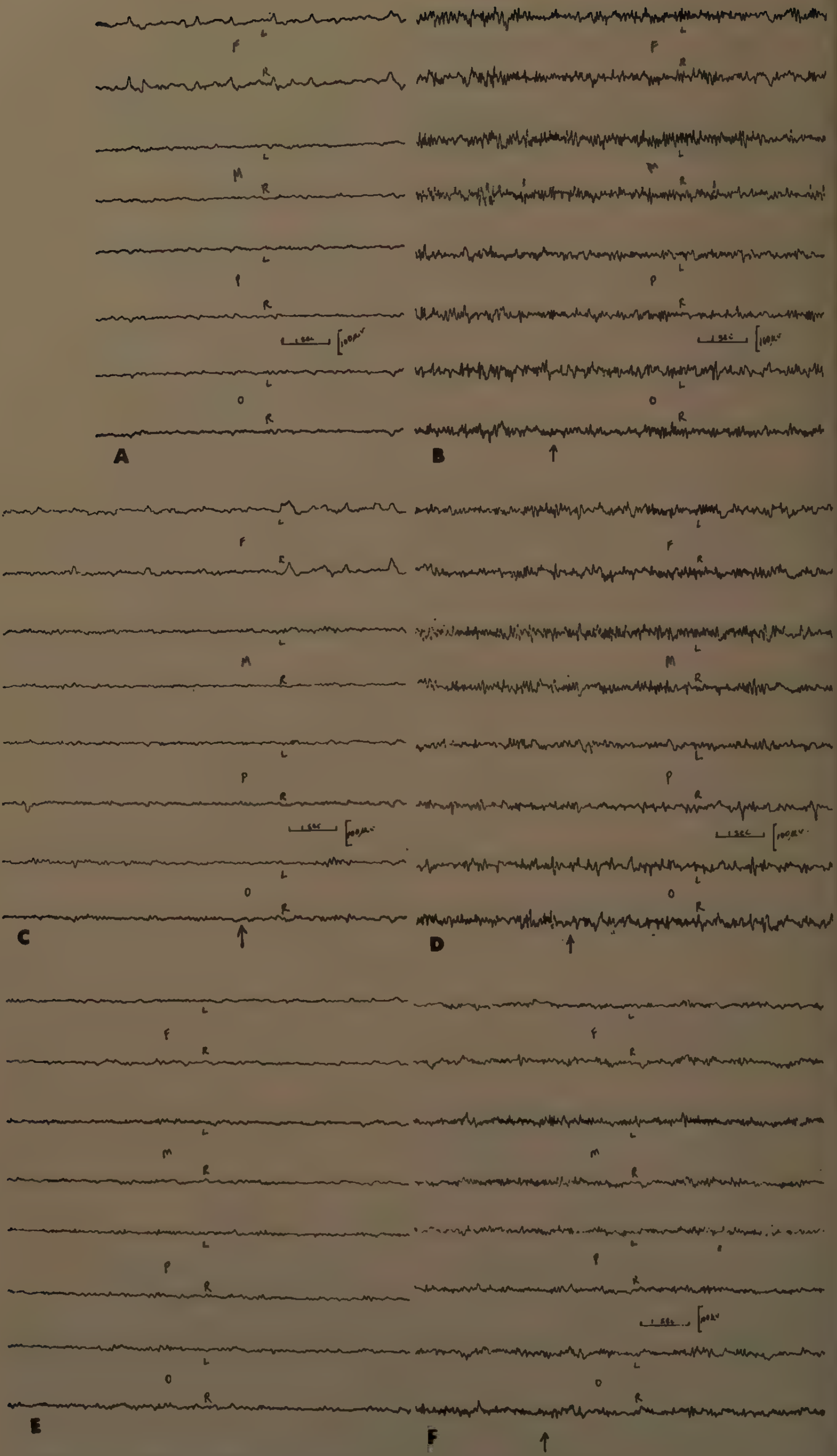

FIGURE 5. Chronic depressive patient, first admitted to hospital in 1937. Readmitted and continuously in the hospital since 1942. (*A*) Normal resting record prior to treatment.

period with phenethylhydrazine. The reawakening of actual schizophrenic symptoms occurred in this patient with the reawakened EEG effect.

The entirely dissimilar effect of iproniazid in a chronic depression of many years' duration is illustrated in FIGURE 5. In this series iproniazid suppresses the normal pretreatment Pentothal effect as it begins to relieve symptoms.

It is noteworthy that these drugs do not themselves produce any clear or characteristic EEG change. The physiological alteration must be brought to light by the Pentothal injection.

These EEG observations are entirely consistent with the clinical observations. The drugs that relieve schizophrenic manifestations and suppress the associated EEG changes, and those that reawaken schizophrenic symptoms also bring back the EEG changes. The preactivation effect illustrated is evident only in schizophrenic reactions. We have progressed to a point that it seems possible to predict the effect of treatment from EEG findings, the most resistant, chronic patients excepted.

Summary

Observations with antidepressant drugs are described and tabulated. Depressed patients (as defined) are significantly susceptible to relief from these drugs, particularly iproniazid, the drug most extensively studied thus far, and also from Tofranil* (G-22355) and, possibly, phenethylhydrazine. Schizophrenic and manic reactions can be aroused or aggravated by these drugs in susceptible patients. Correlation of the drug effects and certain EEG observations indicates that distinction between schizophrenic and depressive psychotic reactions at a physiological as well as at a clinical pharmacological level, is possible. The implication is for a physiological and perhaps neuroanatomical background in psychotic reactions.

References

1. LOOMER, H. P., J. C. SAUNDERS & N. S. KLINE. 1957. Am. Psychiatric Assoc. Psychiatric Research Repts. **8:** 129–141.

Discussion of the Paper

GEORGE A. ULETT (*Department of Psychiatry and Neurology, Washington University, St. Louis, Mo.*): This stimulating paper has an added benefit for me as it brought me a closer knowledge of Goldman's research.

(*B*) Record prior to treatment following injection of Pentothal. Arrow indicates third injection of 100 mg. (300 mg. total). (*C*) Record 3 days later, after the intravenous injection of 50 mg. iproniazid, 120 sec. after the second injection of 25 mg. (*arrow*). This portion of the record is indistinguishable from the resting record in *A* above. (*D*) The effect of pentothal injection. Arrow indicates 120 sec. after the third injection of 100 mg. (300 mg. total), following the intravenous injection of iproniazid. This portion of the record shows no change from the Pentothal effect prior to the injection of iproniazid, and is therefore quite different from the reaction in schizophrenic patients. (*E*) Resting record when patient was receiving iproniazid (50 mg. orally 3 times daily for 5 weeks). This record is practically indistinguishable from previous resting records. (*F*) Tracing after the injection of Pentothal. Arrow indicates 125 sec. after third injection of 75 mg. Pentothal (275 mg. total). This record indicates the effect of Pentothal in a depressed patient while under the influence of orally administered iproniazid. The relatively normal response to Pentothal previously noted is now diminished in somewhat the same fashion that the excessive response to Pentothal in schizophrenic individuals is diminished after medication with an active phenothiazine.

* Geigy.

The science of electroencephalography was fathered by Hans Berger, a psychiatrist and, since his time, psychiatry has been disappointed that the resting electroencephalographic (EEG) tracing has been of little or no use in differentiating among the nonorganic psychoses. However, a number of activation techniques have been used in an attempt to bring out latent covert patterns in the EEG that would be typical for given psychiatric diagnostic categories. Hyperventilation, pentamethylenetetrazol (Metrazol*), photo-Metrazol, sleep, and photic activation are perhaps the most commonly used. Our laboratory has long been interested in these methods, but we found only one positive relationship: that between symptoms of anxiety and fast activity evoked by photic stimulation. Pursuing this lead further, Shagass, in Montreal, Ont., Canada, reported a photic activation index that differentiated between anxiety on the one hand, and depressions, psychotic and reactive, on the other. Using intravenous Amytal and an end point of increased beta activity in the EEG, Shagass has published a series of fascinating papers on what he calls the sedation threshold.[1] Disturbing, however, is the fact that this new index differentiates the psychotic depressions as one group and places together reactive depression and anxiety reaction. Also, Shagass reports that his data do not relate to the diagnostic categories as delineated by the Funkenstein test, which in itself is a controversial diagnostic measure. Now Goldman presents us with yet a new method of EEG barbiturate activation and still another diagnostic grouping. With his technique the depressives fail to respond and the schizophrenics give an abnormal response. The major problem with all of these techniques is, of course, that of the well-recognized difficulties of using clinical diagnoses as the validating criteria. The changes in Goldman's index with clinical improvement and with phenothiazine medication, however, are very interesting. However, I am at a great loss to fit his observations with those of Roth,[2] who injected Pentothal early in the course of electroconvulsive therapy at a time when slow activity had not yet become evident in the resting EEG. Roth found that, if the Pentothal could provoke evanescent abnormal activity, this augured well for the results of shock treatment. I am impressed by the magnitude of Goldman's data and the particularly intriguing theoretical implications for a simpler classification of mental illness, which coincide so well with recent advances in psychopharmacology. The method looks promising and I hope that his findings can be replicated by other workers.

Fink, of Hillside Hospital, New York, N. Y., has published results indicating that the production of overt slow activity by the convulsive therapies is a favorable sign and perhaps even an essential part of the recovery from depression by this method of treatment. It is, therefore, disappointing that therapeutic doses of iproniazid (Marsilid)[3] do not produce slow activity in the EEG. I wonder if, like Roth, Goldman has seen, under Pentothal activation, slow bursts in the EEG of any of the depressed patients recorded early in the course of Marsilid therapy?

In our laboratory we have been trying to block electroconvulsive therapy (ECT) produced EEG slowing by giving sizable (10-mg.) daily doses of atropine to patients on electroshock therapy. In a preliminary study we were able successfully to reduce the amount of delta in the EEGs of schizophrenics by this procedure.[4] In a study currently near completion, however, we have apparently been less successful in blocking the retardation in patients suffering from depression.[5] Such large doses of atropine, administered chronically throughout the course of treatment seem, in our first look at the data, to have no marked effect upon the results of treatment.

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* Bilhuber-Knoll.

Concerning the clinical portion of Goldman's paper I can say little, as my own experience with antidepressant drugs has been limited. The staff at our hospital has been overwhelmed by the welter of new drugs and, as one of my co-workers aptly put it, "we have not yet joined the drug-of-the-month club." Heusler of St. Louis State Hospital, St. Louis, Mo., and I, however, feel that we may have a partial answer to the problem of more rapid clinical drug evaluation, at least in the field of tranquilizers, as we have very recently had some very intriguing data from an attempt to use an electronic, objective method to assess the comparative tranquilizing effectiveness of several of the new ataractics upon the behavior of disturbed patients.[6] We are still pondering ways to turn this type of technique to a more rapid, objective evaluation of the antidepressants.

Goldman's experiences seem to indicate that it is possible to treat safely a large number of patients with Marsilid if one is willing to proceed cautiously with a constant attention and alertness to the known side effects and complications of this drug. I conducted an unofficial hospital-corridor poll among a random sample of about a dozen St. Louis psychiatrists and found that almost without exception they had had some rather dramatic therapeutic responses to Marsilid; however, although none of the physicians questioned had encountered any serious complications himself, all but one had given up the use of the drug because of recent unfavorable publicity.

To my surprise, a similar sampling among internists revealed an almost complete lack of experience with Marsilid. In this regard, it is pertinent to recall the recent excellent papers on endogenous depression by Cassidy *et al.*,[7] Watts and Obst,[8] and Campbell.[9] These authors, with most convincing data, stress the fact that a majority of depressions never reach the psychiatrist, but rather present themselves to the internist and general practitioner with symptoms of what appear to be a variety of somatic illnesses. Possibly here is a vast pool of patients whose somatic symptoms are of central nervous system origin, who could be helped if the general practitioner had a greater familiarity with these amine oxidase inhibitors.

References

1. Shagass, C. 1958. Neurophysiological studies of anxiety and depression. Psychiat. Research Repts. 8, APA. 100–117.
2. Roth, M. 1951. Changes in the EEG under barbiturate anesthesia produced by electro-convulsive treatment and their significance for the theory of ECT action. EEG Clin. Neurophysiol. **3:** 261–280.
3. Fink, M. 1957. Relation of electroencephalographic delta activity to behavioral response in electroshock. A.M.A. Arch. Neurol. Psychiat. **78:** 516–525.
4. Ulett, G. A. 1957. Effect of atropine and scopalamine upon electro-encephalographic changes induced by electro-convulsive therapy. EEG Clin. Neurophysiol. **9:** 217–224.
5. Johnson, L. C., G. A. Ulett, K. Smith & J. O. Sines. 1959. Quantitative studies of electroencephalographic changes induced by electro-convulsive therapy and the effectiveness of atropine in controlling these changes. Am. EEG Soc. 13th Ann. Meeting.

6. Heusler, A., G. A. Ulett & J. Blasques. 1959. Noise level index—an objective measurement of the effect of drugs on the psychomotor activity of patients. Ann. Meeting Am. Soc. Med. Psychiat.
7. Cassidy, W. L., N. B. Flanagan, M. Spellman & M. E. Cohen. 1957. Clinical observations in manic-depressive disease. J. Am. Med. Assoc. **164:** 1535.
8. Watts, C. A. H. & D. Obst. 1957. The mild endogenous depression. Brit. Med. J. 3–8.
9. Campbell, J. D. 1950. Mild manic-depressive psychosis, depressive type: psychiatric and clinical significance. J. Nervous Mental Disease. **112:** 206–236.

THE USE OF JB-516 IN PSYCHIATRY

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During the past eighteen months clinical and laboratory studies have been conducted upon Compound JB-516 (Catron*) at Beth El Hospital through the joint activities of its Department of Neuropsychiatry and Research Laboratory. This general voluntary hospital does not as yet have provision in its wards for the care of active psychotic reactions, but its large outpatient department includes an Adult Psychiatric Clinic and a Child Guidance Clinic. Until recently electroconvulsive therapy was available to ambulatory patients at this hospital, a service that was not offered by any of the other voluntary hospitals in the largest borough of the city of New York. The large number of patients on the rolls of the outpatient department is truly representative of the types of cases handled by the general medical practitioners in the conglomerate community. Any new therapeutic principle that can offer the most good for the greatest number in the shortest time will be accepted, after a thorough investigation and trial for effectiveness and safety, into the hospital armamentarium. During the years since World War II increasingly greater utilization of chemotherapeutic agents has made for more rapid and sustained recovery from some of the most common and distressing of the mental illnesses. In keeping with this trend, an investigative survey was initiated to ascertain the effectiveness of JB-516 upon the depressions that constitute the most frequent of the psychiatric symptom complexes appearing in the clinics.

To determine the acceptability of a pharmaceutical as a therapeutic agent, a number of factors must be considered: its specific effectiveness, promptness of action, lasting action of a single dose, freedom from side action, low toxicity, and relatively low cost; the approach in the study upon JB-516 was in strict conformity with these considerations.

An initial study to ascertain symptomatic response was conducted upon 8 hospitalized patients in the medical and surgical wards who exhibited some degree of depression. Within 1 week, upon a daily dosage of 50 mg., all responded favorably, as evidenced by increasing alertness, diminution of apathy or confusion, greater participation in ward routine, and better amenability to their management. This was quickly recognized and recorded by the nursing staff. Dosages were then gradually reduced over a 30-day period, the improvement persisting and being maintained for 2 to 4 weeks even after the medication was discontinued. This clearly indicated that JB-516 was specifically effective as an antidepressant and that its action was appreciably rapid and lasting.

Toxicity studies were thereupon carried out on 16 patients over a 3-month period. These studies included liver profiles, hemograms, urine determinations, and other studies. Electrocardiography and electroencephalography were performed. My colleague, I. J Greenblatt, presents the results of these studies, as well as of later intensive investigations elsewhere in this monograph. No changes in cephalin flocculation, thymol turbidity, or urinary urobilin were observed. All of the other studies evoked similarly normal findings. In dosages of more than 50 mg./day, low blood-pressure readings, with accompanying lassitude and fatigability, became quickly apparent. In combination with phenothiazine preparations (promazine hydrochloride—Sparine*) JB-516 in doses of 75 mg. per day, especially in the older-age group, would place a patient in deep sleep, lasting sometimes for 16 hours. Exhaustive titrations for potency led to the conclusion that the safest daily dose yielding the most satisfactory clinical response was 50 mg. or less.

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* Lakeside.

A preliminary clinical study was then conducted upon 30 ambulatory patients over a 3-month period. These patients ranged in age from 22 to 71 years, of both sexes, and all exhibited depressions of varying degrees and types. They were started on dosages of 50 mg./day, and reduced to 25 mg./day within 5 days as improvement occurred. With continuing improvement, the dose was reduced to 12.5 mg./day and was maintained at this level for 30 days; the dose was then leveled off to 6.25 mg./day. Concomitant blood determinations for liver profile, hemograms, urine examinations, and electroencephalography were done. All of the toxicity studies were negative. Very gratifying clinical responses were obtained, characterized by elevation of mood, diminution of confusion, reduction in depressive attitudes, alertness, and ability to maintain work and social adjustments.

TABLE 1
CLASSIFICATION OF SUBJECTS

Type	No.	Sex	Age groups
Reactive (psychoneurotic) depression	13	F	18 to 58 years
	8	M	31 to 50 years
Involutional depression	5	F	47 to 61 years
	1	M	50 years
Post partum depression	1	F	22 years
Cyclothymic depression	4	F	39 to 49 years
	1	M	45 years

It was after this gratifying preliminary survey that a more intensive and critical study over a 9-month period was conducted with JB-516 upon 33 ambulatory nonhospitalized patients exhibiting various types of chronic depressions. Twenty-three were females and 10 were males, and the age range was 18½ to 61½ years. The diagnostic categories were: psychoneurotic (reactive) depressions, 21; involutional depressions, 6; cyclothymic depressions, 5; *post partal* depression, 1. All except the 22-year-old patient with *post partal* depression had had prior active psychiatric treatment of 1 or more forms for 2 years or more, including psychotherapy, chemotherapy, electroconvulsive therapy, and mental hospital residences.

TABLE 1 illustrates the diagnostic classification of the subjects, broken down into numbers, sexes, and age groups.

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* Wyeth.

TABLE 2 illustrates the duration of the disease categories broken down into number, sexes, and time in years.

TABLE 3 illustrates, for each category, prior electroconvulsive therapy, institutionalization, and chemotherapy. All the patients, except the one with post-partal depression, had the benefit of psychotherapy (nonanalytic) in addition to one of the other forms. Chemotherapy included the amphetamines, phenothiazines, reserpine, meprobamate (Equanil*), cholinergic agents, and combinations of cholinergic and adrenergic agents; one patient in the involutional group had iproniazid.

TABLE 2
DURATION OF DISEASE

Type	No.	Sex	Duration
Reactive (psychoneurotic) depression	13	F	2 to 12 years
	8	M	2 to 10 years
Involutional depression	5	F	2 to 4 years
	1	M	2 years
Post partum depression	1	F	2½ months
Cyclothymic depression	4	F	3 to 13 years
	1	M	2 years

TABLE 3
TREATMENT PRIOR TO JB-516 (CATRON)

Type	ECT	Hosp. and ECT	Chemotherapy
Reactive (psychoneurotic) depression	2 M 3 F	1 M 1 F	8 M 12 F
Involutional depression	1 M 5 F		5 F
Cyclothymic depression	2 F	1 M 2 F	4 F
Post partum depression			1 F
Total	13	5	30

Each patient was advised to discontinue all prior medication. No build-up was given for the new pharmaceutical. The patient was advised that a new medication would be given, that it was not as yet available commercially, and that periodic blood tests, blood pressure determinations, electroencephalography, and other necessary procedures would be performed. A 2-weeks' supply of the medication was provided. The initial dose was 25 mg./day. This dose was maintained for 2 weeks. Blood was then drawn for liver profile studies, a general physical examination, including blood pressure determinations, was made, and the patient was interviewed regarding his or her progress. If appreciable improvement had occurred, the dose was lowered to 12.5 mg./day. This procedure was repeated at 2-week intervals, or sooner if necessary. After

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* Wyeth.

1 month the daily dose was 6.25 mg. Whether the dosage was maintained at the initial level or at a higher or a lower level depended upon the patient's progress. In other words, manipulation of the dosage was accomplished on an individual basis, depending upon the responsiveness or the appearance of side effects. In every case where improvement had supervened the dose was 3 mg./day after the sixth week. After the eighth week the patient was advised, if improvement continued, to take the pill every second or perhaps every third day, depending upon progress. Auxiliary medication was utilized where indicated; if at 3 mg./day dosages the patient was elated or unduly active, reserpine (Serpasil*) 0.1 mg. was prescribed twice or three times daily. On several occasions Dexedrine† sulfate .005 each morning or Dexedrine .010 Spansules each morning was prescribed to patients who needed an extra "push" at high dosage levels of JB-516.

The psychotherapeutic effects of treatment with JB-516 (Catron) are outlined in TABLE 4, which summarizes the psychotherapeutic responses of patients in all categories, as indicated by marked improvement, improvement, and ineffectual.

TABLE 4
PSYCHOTHERAPEUTIC EFFECTS OF JB-516 (CATRON)

Type	Marked improvement	Improved	Ineffectual
Reactive (psychoneurotic) depression*	12	5	3 (21)
Involutional depression	3	3	0 (6)
Post partum depression	1	—	0 (1)
Cyclothymic depressive reaction	2	2	1 (5)

* One doubtful response.

It will be noted that 60 per cent of the patients in the psychoneurotic group showed a marked improvement, 15 per cent were improved, and 9 per cent were not improved. In the involutional group all patients were improved, one half of these showing marked improvement. Of the 5 patients in the cyclothymic group, 2 showed marked improvement, 2 were improved, and 1 was not improved. Altogether, approximately 79 per cent of the patients showed improvement ranging from mild to marked.

Toxicity studies were consistently negative. Electroencephalography did not reveal any alteration of the before-and-after patterns in 85 per cent of the cases; in the remaining 15 per cent the pattern obtained after therapeutic response was characterized by a slowing of the frequency and an increase in amplitude.

FIGURES 1 and 2 illustrate the before-administration and after-administration tracings in those instances in which alterations occurred.

A number of side effects were observed. The most frequent was hypotension, which occurred in 20 per cent of all patients, particularly in those with more than 150 systolic before treatment, and this was frequently manifested by dizziness, weakness, and faintness; on 2 occasions patients fell and were bruised. Manipulation of the dosage or addition of Dexedrine or neostigmin (Prostigmin*) quickly proved effective. Constipation was the complaint of 4 patients who had been on JB-516 for more than 3 months and who exhibited marked improvement; this complaint was rather distressing, but it was managed without very much difficulty by the use of mineral oil and milk of magnesia mixtures and Prostigmin. Two patients suffered with dysuria, but this symptom quickly abated when the dosage of JB-516 was lowered. One patient, a 37-year-old male with reactive depression who exhibited dramatic improvement, developed ankle edema; this condition responded promptly to Neohydrin.† One female patient, 54 years of age, in the involutional group, a severe diabetic of long standing in whom mental illness was more severe and had been resistant to all efforts at psychiatric treatment for more than 4 years and who

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* Ciba.
† Smith, Kline & French.

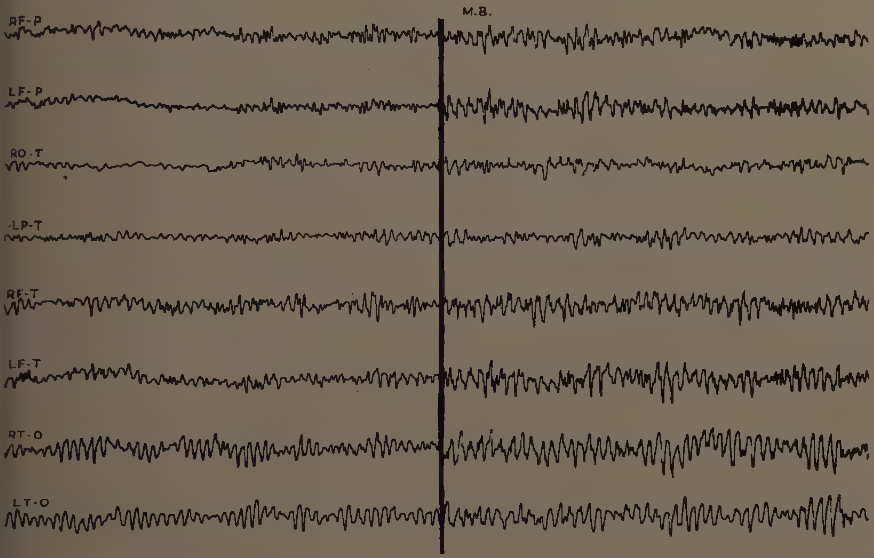

Figure 1. EEG tracings (*left*) before and (*right*) after the administration of JB-516 (patient M. B., aged 51 years).

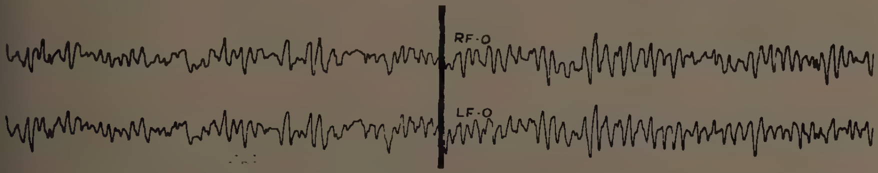

Figure 2. EEG tracings (*left*) before and (*right*) after the administration of JB-516 (patient C. G., aged 39 years).

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* Roche.

† A preparation containing 3-chloromercuri-2-methoxypropylurea (Lakeside).

exhibited a most dramatic improvement, was stricken with hepatitis in the ninth week of treatment. The recently described Morrison test on her blood returned a positive agglutination (1–16) with rhesus monkey blood, indicative of viral hepatitis. Her condition proved to be irreversible. Greenblatt reports at length regarding the results of the laboratory procedures performed upon her elsewhere in these pages.

Summary

After initial potency titrations, toxicity studies, and a gratifying preliminary clinical survey, an extensive and critical clinical study over a 9-month period was conducted with the monamine oxidase inhibitor compound JB-516 (Catron) upon 33 ambulatory nonhospitalized patients were exhibited various types of chronic depressions. Twenty-three were females and 10 were males, and the age range was 18½ to 61½ years. The diagnostic categories were: psychoneurotic (reactive) depressions, 21; involutional depressions, 6; cyclothymic depressions, 5, and post-partal depression, 1. All except the 22-year-old patient with post-partal depression had had prior active psychiatric treatment of 1 or more forms for 2 years or more, including psychotherapy, chemotherapy, electroconvulsive therapy, and mental-hospital residences. Periodic blood determinations for liver profiles and electroencephalography were performed. An increase in EEG amplitude and slowing of frequency occurred in 15 per cent of the patients after therapeutic response. Side effects, which occurred in a small percentage of the cases, included hypotension, constipation, and dysuria, and 1 patient with a severe diabetes was stricken with viral hepatitis. Seventy-nine per cent of the patients exhibited improvement ranging from mild to marked. Sixty per cent of the patients in the psychoneurotic group showed a marked improvement, 15 per cent were improved, and 9 per cent were not improved. In the involutional group all patients were improved, one half of these showing marked improvement. Of the cyclothymic group (5 in all), 2 showed marked improvement, 2 were improved, and 1 was not improved.

Discussion of the Paper

NATHAN S. KLINE (*Rockland State Hospital, Orangeburg, N. Y.*): I congratulate Henry Agin on his careful study and results, which are very similar to our own. I have no quarrel with his data.

Agin mentioned using doses of 50 mg. It is always encouraging to have someone use high doses, for thus one has precedent for doing so oneself. He also stated that he used 25 mg. in the second phase of his study. I wonder what he considers the best starting dose, and whether one can begin with a dosage of 50 mg. and build it up more rapidly, or whether 25 mg. appears to be optimal.

I may mention that we have frequently found that patients tend to be fairly demanding and needful of reassurance during the first weeks of treatment.

Agin also mentioned that he sometimes used reserpine, apparently for its sedative or ataractic action. In contrast, we have found this drug to be a further stimulant on the occasions when we have employed it. Obviously, either we are using different patients or one of us is looking at something different from what the other sees.

My final point is that if edema, which we see rather frequently, becomes disturbing, the addition of chlorothiazide (Diuril*) almost always enables one to continue the medication in the regular dosage.

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* Merck Sharpe & Dohme.

TREATMENT OF HOSPITALIZED MENTAL PATIENTS WITH IPRONIAZID*

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Introduction

This study was designed to assess the clinical efficacy of iproniazid (Marsilid†) in the treatment of depressive states and chronic schizophrenia occurring in a group of hospitalized patients. Iproniazid therapy has been reported upon both favorably[1-10] and unfavorably[11-18] in a number of different studies.

While the evaluation of a drug in only 50 patients could hardly upset the balance of opinion either way, any additional evidence concerning the efficacy of iproniazid was deemed desirable for the following reason: the urgent need for a compound effective in the treatment of depressive states and in schizophrenic reactions refractory to tranquilizer therapy demanded continued trials with the drug to substantiate or refute adverse publicity that might prejudice its use. Furthermore, we at the Topeka State Hospital have been assessing experimental compounds for a number of years and, as a result, we have attained a level of psychopharmacological sophistication and experience that warranted our undertaking the study of a potentially harmful compound.

Selection of Patients

With the exception of 2 patients with organic brain disease, the test group was almost evenly divided into 2 disease categories: schizophrenic reactions and depressions. The age range of the patients was 26 to 54, with an average of 44 years and an average chronicity of illness of 7 years. Females predominated in a ratio of 3.5:1. The detailed breakdown of the group is shown in TABLE 1.

The individuals with schizophrenic reactions could be characterized as withdrawn, regressed, apathetic, seclusive, inactive, and autistic. The patients with depressive symptomatology fell into the several categories of depressed states. The average age and chronicity of illness strongly suggest that this was a prognostically poor group of patients. This opinion is confirmed by the failure of many of these patients to respond to prior somatic therapy (electroconvulsive or insulin therapy) and chemotherapy (phenothiazines, meprobamate, and reserpine).

Method

Evaluators. Twenty-three residents in training in psychiatry were each assigned to only one or to very few patients receiving iproniazid. The use of a large number of evaluators obviated the possibility that results might become excessively colored by the rater bias of any single investigator.[19] It also en-

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* The work reported in this paper was supported in part by a grant from Hoffmann-La Roche Inc., Nutley, N. J. which also provided the iproniazid (Marsilid) used.

† Hoffmann-La Roche.

sured that each patient would be under constant clinical and laboratory scrutiny.

Laboratory studies. Prior to the onset of iproniazid therapy, the following battery of laboratory tests was performed: indirect van den Bergh; alkaline phosphatase (King-Armstrong units); thymol turbidity; cephalin flocculation (24 hours); blood urea nitrogen; nonprotein nitrogen; complete urinalysis including albumin, acetone, sugar, and urobilinogen; and red blood cell, white cell, and differential count and hemoglobin. These tests were repeated weekly for 4 weeks and then bimonthly for the remainder of the study.

Therapy observations and precautions. Patients were carefully observed for any signs of untoward reaction. Blood pressure readings were recorded 1 or 2 hours after medication. Physicians were particularly alerted to the possibility of the development of (1) icterus; (2) premonitory signs of agranulocytosis (sore throat, fever of undetermined origin, or lesions of mucous membranes); or (3) the development of clinical symptoms or laboratory evidence of renal or hepatic dysfunction. Any of these were to be considered adequate cause for discontinuing iproniazid.

TABLE 1
COMPOSITION OF TEST GROUP

Classification	Number of patients	Average age (years)	Average duration of illness (years)	Sex (M/F)
Schizophrenic reactions	26	41	11	7/19
Undifferentiated type	13	43	12	5/8
Paranoid type	8	42	13	1/7
Catatonic type	3	26	3	1/2
Hebephrenic type	2	45	14	0/2
Depressions	22	48	3	3/19
Manic-depressive, depressive phase	5	52	3	2/3
Involutional	1	54	2	0/1
Depressive reaction	10	52	3	0/10
Psychoneurotic	6	38	5	1/5
Organic brain disease	2	45	4	1/1
Total	50	44	7	11/39

All untoward reactions, as well as changes in mental status, were promptly recorded so that they could be correlated with dosage level and duration of treatment.

Concomitant therapies. Pre-existing programs of therapy were maintained. In those instances in which patients were receiving psychotherapy, this was continued. Adjunctive therapy activities were maintained or increased up to and within the needs and capabilities of the patients.

Dosage. Patients were routinely started on 50 mg. iproniazid, orally 3 times daily. After 2 to 4 weeks the dosage was gradually and progressively reduced, with the final goal of a maintenance dosage of about 25 mg./day.

If a patient showed evidence of toxicity or untoward reaction, the dosage was promptly reduced or discontinued. Likewise, in instances where patients showed early and favorable responses to iproniazid, the dosage was reduced prior to 2 to 4 weeks.

Auxiliary medications were not prescribed on a routine basis, nor was pyridoxine incorporated into the therapeutic regime. In cases of insomnia, various barbiturates were added; in a similar way, Dexedrine* was prescribed for dizziness and mild hypotension, and diuretics for edema.

Method of evaluation. A behavioral rating scale that includes 24 items covering the gamut of patient behavior was used.[20] Residents evaluated each of these items on a 4-point scale: no, slight, moderate, and marked improvement. Items that were not applicable to a given patient were not rated, so that a rating of "no improvement" for any of the 24 items implied that the patient had some deficiency that had failed to respond in some degree to iproniazid therapy.

I then assessed these individual item ratings and converted them into an over-all improvement rating (for each patient) on a 6-point scale: none, slight, slight-to-moderate, moderate, moderate-to-marked, and marked. This final evaluation was based on a composite of the evaluations of the individual items.

In those patients in whom iproniazid was discontinued prior to the completion of the project, the estimate of the degree of improvement was based upon an assessment of the patient's mental status within one week of the time of termination of treatment.

For the purposes of reporting this project, only moderate or marked improvements were considered significant.

Results

The duration of treatment varied widely in different patients because, for various reasons, medication was discontinued prior to completion of the project in 31 of the 50 patients. The average duration of treatment for the entire group was 83 days. In 23 of the 31 cases medication was discontinued after an average of 56 days of treatment (7 to 213 days) for 1 of 3 reasons: (1) dissatisfaction with patient's progress, (2) worsening of mental state, or (3) development of toxic signs.

Seven of the patients were discontinued after they had shown significant improvement in order to prepare them for release from the hospital (average duration of treatment, 69 days). One patient was discontinued for nonmedical reasons after 51 days of treatment. Eleven patients in the series were treated for less than 30 days, and their treatments were discontinued because of: development of hypomanic state; anorexia, persistent and severe, with weight loss; dizziness; worsening of mental state; insomnia; edema; or hypotension, severe and persistent. In most instances more than one of these types of untoward reaction contributed to the decision to discontinue medication. At the close of the project, in the 19 patients still receiving iproniazid the average maintenance dosage was 75 mg./day.

Response evaluation. TABLE 2 lists the criteria of evaluation in the order of increasing effectiveness of response to iproniazid therapy. It is clearly apparent that certain aspects of the patient's psychopathology remain unaltered as a result of this treatment.

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* Dextro-amphetamine sulfate (Smith, Kline & French).

Defective memory, absence of insight, defective orientation, the tendency toward self-mutilative behavior, poor judgment, combativeness, and compulsivity are uninfluenced by iproniazid.

Hypoactivity, tension, hallucinations, mannerisms, negativism, insomnia, hostility, delusions, defective realistic planning, personal hygiene, and appropriateness of conversation are at times favorably influenced by iproniazid, and there is an even chance that amicability, sociability, affect, participation in adjunctive therapy, accessibility, and appetite will be favorably influenced.

When these same criteria are evaluated in only those patients who have been rated to have shown significant over-all improvement, the order of increasing effectiveness of response to iproniazid remains essentially unchanged, but the frequency of favorable response of the individual criteria increases substantially.

TABLE 2

CRITERIA OF EVALUATION RESPONSES

Factor	Significant degree of improvement (percentage of patients)		Factor	Significant degree of improvement (percentage of patients)	
	Total group	Significantly improved group		Total group	Significantly improved group
Memory	0	0	Hostility	21.7	66.7
Insight	0	0	Delusions	25.0	75.0
Orientation	0	0	Realistic Planning	26.8	78.7
Self mutilation	0	0	Dress	28.5	75.0
Judgment	3.5	25.0	Appropriateness of conversation	35.5	85.7
Combativeness	4.5	25.0			
Compulsivity	7.1	0	Amicability	46.5	100
Hypoactivity	12.0	25.0	Sociability	52.0	100
Tension	13.5	40.0	Affect	52.1	100
Hallucinations	15.0	49.8	Participation in adjunctive therapy	53.1	100
Mannerisms	17.2	62.5			
Negativism	18.0	60.0	Accessibility	53.2	100
Sleep	21.2	85.8	Appetite	56.7	100

A composite picture of the patient who has responded to iproniazid (favorably) might be one characterized by: (1) disappointing improvement in memory, insight, orientation, compulsivity, and self-mutilative tendencies; (2) occasional improvement in judgment, level of physical activity, tension, and hallucinations; (3) fairly constant improvement in negativism, mannerisms, hostility, personal hygiene, delusions, realistic planning, sleep patterns, and appropriateness of conversation; and (4) very consistent improvement in participation in adjunctive therapy, accessibility, affect, appetite, sociability, and amicability.

General therapeutic responses. Clinical improvement in those patients who responded favorably to iproniazid was usually noted first between the seventh and tenth days after medication was started. Seventeen patients in the series

(34 per cent) showed a significant response to iproniazid therapy. The improvement rate in the patients with schizophrenic reactions was 34.6 per cent and in those with depressions, 36.3 per cent. Twenty-five patients (50 per cent) have either been released from the hospital or are awaiting release. The release rate is in excess of the improvement rate, inasmuch as 8 patients whose over-all improvement was not rated as significant have been released from the hospital.

These 8 patients did show improvement in the areas of amicability, sociability, and accessibility and, although their over-all improvement was not rated as significant, the particular changes that were effected in their status were conducive to their release. Treatment of one of these 8 patients was discontinued after 51 days because of adverse newspaper publicity and, upon assessment, appeared to be only slightly improved. However, he continued to make progress without further medication and was released 1 month later. A summary of the results is shown in TABLE 3.

TABLE 3
RESULTS: CLINICAL IMPROVEMENT

Classification	Significantly improved		Released	
	Patients	Percentages	Patients	Percentages
Schizophrenic reactions	9	34.6	12	46.2
Depressions	8	36.3	13	59.1
Manic depressive–depressive state	2	40	2	40
Depressive reaction	3	30	7	70
Psychoneurotic	3	50	4	67
Total	17	34	25	50

In those patients in whom a favorable response was elicited, the usual pattern was one of progressively increasing interest and involvement in their environment, the physical aspects of this reinvolvement often being preceded by a renewal of interest in surroundings, people, and social activities. Apathy and depression were replaced by improved affect and interest in living.

Occasionally, remarkable amelioration of hallucinations and delusions was noted. Sometimes, despite only minimal decrease in the intensity of the hallucinations or delusions, the emotional repercussions to them were considerably attenuated, and the patient assumed a healthier or more tolerant attitude toward his mental state.

Untoward effects. Twenty-four of the patients (48 per cent) exhibited one or more side effects when they were on the 150-mg./day dosage schedule. These side effects, listed in the order of decreasing incidence, are shown in TABLE 4. The difference in incidence between the 150-mg./day level and the 75-mg./day level is striking.

The most common side effect encountered was pedal edema, which occurred in 11 patients. In most cases it was bilateral and occurred as early as the second day of treatment or as late as the forty-fifth day. Untreated, the edema persisted as long as 2 weeks, eventually subsiding spontaneously; amelioration could be accelerated by prescribing bed rest and diuretics or by discontinuing iproniazid. In 3 of these patients iproniazid was discontinued (sixth, twelfth, and fourteenth days of treatment) because of an associated albuminuria.

Anorexia was the second most frequent side effect. It occurred in 6 patients, all at the 150-mg./day level, and was attended by a weight loss of 11 to 18 pounds. Marked weight loss, in all 6 instances, prompted the decision to stop medication.

Increased tension occurred in 5 patients. Three of these patients exhibited both subjective and objective evidence of discomfort, apprehension, and an ill-defined sense of impending disaster. In 1 instance this tension subsided spontaneously and, in the 2 others, after iproniazid was discontinued. The last 2 cases went on to a frank hypomania, 1 of them subsiding when iproniazid was discontinued and the other responding promptly to electroconvulsive therapy.

TABLE 4
INCIDENCE OF SIDE EFFECTS

Side effect	Number of instances of side effects	
	At 75 mg./day	At 150 mg./day
Pedal edema	2	9
Anorexia	—	6
Increased tension	1	4
Dizziness	1	3
Insomnia	—	3
Hyperhidrosis	—	2
Hypotension	1	—
Blurred vision	—	1
Headache	—	1
Ataxia	—	1

Mild hypotension was the rule rather than the exception. In all but one case it consisted of a drop in systolic pressure of less than 20 mm. of mercury, was asymptomatic, and returned to permanent normal levels within 1 week. In one instance the blood pressure drop was more profound (84/46), persistent, and associated with severe vertigo. Iproniazid was finally discontinued on the twenty-sixth day of treatment.

No evidence of hepatitis or agranulocytosis was encountered in this series.

Abnormal laboratory findings were frequent, unpredictable, at times inconsistent in their recurrence, and rarely correlated with the clinical picture. The exception to this seemed to be the correlation between pedal edema and albuminuria.

Discussion

An improvement rate of 34.6 per cent in the depressive states is far from remarkable, although it must be kept in mind that these depressed patients had failed to respond to the conventional antidepressive measures; at the start of the project they had an average chronicity of illness of 3 years.

The results with the schizophrenic group (improvement rate of 36.3 per cent), although similar to those obtained in the depressive group, are more noteworthy in that these patients were very clearly chronic, prognostically poor, and treatment-refractory.

A serious defect in the design of this project, not apparent when it was conceived, was the failure to anticipate the relative toxicity of 150 mg. iproniazid per day as compared with one half that amount. It would have been most interesting to see if starting at a lower dosage level would not have yielded equally good therapeutic results without the attendant toxicity that was encountered.

Summary

After being treated with iproniazid for an average of 83 days, 50 chronically ill hospitalized patients showed a significant improvement in 34 per cent and side effects in 48 per cent.

One half of both the schizophrenic and the depressive patients improved with iproniazid therapy to the point where their release from the hospital was feasible.

The results obtained are, in general, similar to those obtained by other investigators, who concluded that iproniazid was an effective agent in the treatment of depression and chronic schizophrenia.

The data strongly suggest that a daily dosage of 150 mg. of iproniazid is attended by a high incidence of untoward side effects and that the incidence of toxicity at the 75-mg./day level is substantially lower and more compatible with the concept of a nontoxic, efficacious agent.

Iproniazid is effective in the treatment of depressions and anergic schizophrenia; in favorable cases improvement usually occurs between the seventh and tenth days of treatment. Little further improvement is noted in patients who have not shown improvement by the third or fourth week of treatment.

In favorable cases, amicability, sociability, affect, participation in adjunctive therapy, accessibility, and appetite are most often changed for the better, but even in these cases the responses of memory, insight, orientation, judgment, and compulsivity are disappointing.

No clinical evidence of hepatic, hematopoietic, or renal dysfunction was noted. The side effects encountered could be dealt with, safely, by alteration of dosage or appropriate auxiliary medication. The variety of untoward effects encountered was less than has been reported by other investigators.

In cases responding to iproniazid therapy, the particular areas of behavior that improve (energy level, speech, accessibility, amicability, affect, and appetite) are those areas where change for the better is conducive to a high release rate.

The results obtained suggest that iproniazid therapy is of value in the treatment of chronic schizophrenia and depressive states.

References

1. Ayd, F. J. 1957. A preliminary report on Marsilid. Am. J. Psychiat. **114:** 459.
2. Breitner, C. 1958. Marsilid in catatonic schizophrenia. Am. J. Psychiat. **114:** 941.
3. Crane, G. E. 1956. Further studies on iproniazid phosphate. J. Nervous Mental Disease. **124:** 322.

4. Ferreira, A. J. D. & H. Freeman. 1958. A clinical trial of Marsilid in psychotic depressed patients. Am. J. Psychiat. **114:** 933.
5. Lauer, J. W., W. M. Inskip, J. Bernsohn & E. A. Zeller. 1958. Observations on schizophrenic patients after iproniazid and tryptophan. A. M. A. Arch. Neurol. Psychiat. **80:** 122.
6. Kamman, G. R., J. G. Freeman & R. J. Lucero. 1953. The effect of 1-isonicotinyl 2-isopropyl hydrazide (11H) on the behavior of long-term mental patients. J. Nervous Mental Disease. **118:** 391.
7. Robie, T. R. 1958. Marsilid in depression. Am. J. Psychiat. **114:** 936.
8. Scanlon, W. G. & W. M. White. 1958. Iproniazid: Its use in office treatment of depression. Am. J. Psychiat. **114:** 1036.
9. Schopbach, R. R. 1958. Clinical note concerning iproniazid. Am. J. Psychiat. **114:** 838.
10. Smarr, E. R., H. Wolf & M. D. Pressman. 1958. Experience with Marsilid with report of one death. Am. J. Psychiat. **114:** 1115.
11. Bennett, I. F., D. Cohen & E. Starer. 1954. Isoniazid in the treatment of the chronic schizophrenic patient. Arch. Neurol. & Psychiat. **71:** 54.
12. Block, R. G., A. S. Dooneief, A. S. Buchberg & S. Spellman. 1954. The clinical effect of isoniazid and iproniazid in the treatment of pulmonary tuberculosis. Ann. Intern. Med. **40:** 881.
13. Crane, G. E. 1956. The psychiatric side effects of iproniazid. Am. J. Psychiat. **112:** 494.
14. Hoshino, A. & E. Cease. 1958. Iproniazid phosphate in the treatment of the chronic hospitalized schizophrenic. Am. J. Psychiat. **114:** 1111.
15. Lemere, F. & J. H. Lasater. 1958. Deaner, a new cerebral stimulant for the treatment of neurasthenia and mild depressions: A preliminary report. Am. J. Psychiat. **114:** 655.
16. Smith, J. A. 1953. The use of the isopropyl derivative of isonicotinyl hydrazine (Marsilid) in the treatment of mental disease. Am. Practitioner. **4:** 519.
17. de Verteuil, R. L. & H. E. Lehmann. 1958. Therapeutic trial of iproniazid in depressed and apathetic patients. Can. Med. Assoc. J. **78:** 131.
18. Wiedorn, W. S. & F. Erwin. 1954. Schizophrenia-like psychotic reactions with administration of isoniazid. A. M. A. Arch. Neurol. Psychiat. **72:** 321.
19. Feldman, P. E. 1956. The personal element in psychiatric research. Am. J. Psychiat. **113:** 52.
20. Feldman, P. E. 1957. Clinical evaluation of chlorpromazine therapy for mental illness. J. Clin. Exptl. Psychopathol. **13:** 1.

Discussion of the Paper

John C. Saunders, (*Rockland State Hospital, Orangeburg, N. Y., and College of Physicians and Surgeons, Columbia University, New York, N. Y.*): When a new form of treatment is developed, there is usually too little initial concern with theory because the methods for drugs produce results so rapidly that major interest focuses upon content. Usually, many participants are engrossed in clinical work, while other potential areas of study remain relatively dormant. The history of iproniazid as an amine oxidase inhibitor in the treatment of the depressive syndrome, however, has progressed from description to the formulation of theories. In fact, I suggested the application of amine oxidase inhibitors to psychiatry in 1955[1] on the theoretical basis of their action on amine metabolism in the brain, with the probability that they would alleviate depressions.

After more than two years' experience, the significant problems with amine oxidase inhibitors are more clearly presented, but the first problem is still present: namely, to learn which amines undergo metabolic changes as a result of these inhibitors and, specifically, which amines produce the psychic changes. This specificity of action will also assist in differentiating some of the psychoses that are alleviated by amine oxidase inhibitors. Amine oxidase is widely distributed in the body and is usually located where true cholinesterase levels are low. In the central nervous system the lenticular nucleus, the hypothalamus, and both pyramidal tracts contain large amounts of this enzyme. Knowledge is now sufficient for experimentation designed to obtain specific data. Some areas, however, remain largely unexplored, and a great part of the delay in these regions is a result of "errors due to propaganda, politics and bureaucracy."[2]

It appears from time to time that the popular press, regrettably, is interested in the actions of drugs only in terms of the all-or-none concept; drugs are either perfect or so intensely toxic that they should not be administered to man. I hope my comments will point out the inestimable value of the so-called side effects of drugs in giving us an understanding of etiological factors in neuropsychiatric diseases.

Reserpine has been a unique research tool, as well as a therapeutic agent, in elucidating some of the pathological processes in several disease entities. Initially used in the treatment of hypertensive cardiovascular disease, reserpine not only reduced blood pressure but, in the words of Robert W. Wilkins, "tranquilized the patients."[3] When this point was called to my attention by Wilkins, and after reading reports in the Indian literature, I was impressed with the need to initiate* a clinical study of reserpine in various neuropsychiatric diseases. Five months of clinical trial showed that reserpine obviously had a tranquilizing action in schizophrenics and affected psychopathological processes. The acceleration of these studies and the publications of many other investigators confirm this observation.

In a few patients, a side action due to the use of reserpine during the treatment of both psychotics and hypertensives was the association of a state of depression; this indicated another path for research in psychiatry. Biochemical studies in both animals and man indicate a significant reduction in serotonin, norepinephrine, and other sympathomimetic amines following reserpine therapy. If the oxidation of these compounds was inhibited, might it not be possible to alleviate this depressive syndrome? On the basis of previous reports, the amine oxidase inhibitor iproniazid (Marsilid) was suggested.[4] As this idea did not appeal to psychiatrists because of their orientation away from an organic basis for the depression, personal initiation of these studies was necessary. Our results were reported,[2, 4, 5] and we are today looking forward to more studies in this area of metabolism and its clinical application.

We have much to learn about the application of amine oxidase inhibition, because we are still unaware of its actual sites of action and the metabolic reactions iproniazid must undergo in order to produce its clinical effects. Some of the factors determining biophysico-chemical structure are probably related to chelate formation with copper,[6, 7] others through competition with pyridoxine nucleotide formation.[8] These and other actions[2, 5] will continue to make the study of iproniazid essential.

One of the several unexplained problems is the property of iproniazid that causes some schizophrenic patients to have an exacerbation of signs and symptoms resembling an acute illness. In a recent publication[9] it was stated that, if a nonpsychotic state is to exist, a dynamic equilibrium must exist between oxidative deamination and transamination of amines in the central nervous system. In discussing the role of iproniazid, it was again emphasized that a dynamic biochemical equilibrium must be established for the sympathomimetic amines produced by these enzymatic reactions.[10] This brings us into an area where there is a demand for amine oxidase inhibitors with a high specificity for a given amine within a neurological system and for a new concept of psychosis.

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* As Senior Research Associate, Ciba Pharmaceutical Products, Inc., Summit, N. J., at that time.

In addition to the adult schizophrenics, it is necessary that we regard childhood (infantile autistic) schizophrenia as a result of an error of metabolism, that is, an excess production of norepinephrine. The chromaffin system arises from ectoderm buds which, in the early stages of development, form the adrenal medulla and accessory chromaffin organs found in the retroperitoneal tissue (organ of Zuckerkandl) and near the sympathetic ganglia (paraganglia). In mammals, the most prominent of these small masses of chromaffin cells outside the adrenals is Zuckerkandl's aortic body, situated near the root of the inferior mesenteric artery. A few biochemical studies on adrenergic neurons show that amine formation in chromaffin tissue and in sympathetic neurons constitutes a similar synthesis. We must remember that areas of the brain that perform autonomic and possibly psychic functions also contain a greater concentration of these sympathomimetic amines. Studies reviewed presented evidence that the central functions of the sympathomimetic amines are similar to this action on peripheral adrenergic nerves. These amines are powerful stimulants of the ascending activating system of the reticular formation.[11] The adrenal gland of newborn calves contains a preponderance of norepinephrine.[12] A detailed study by Shepherd and West[13] of this changeover from norepinephrine to epinephrine during growth resulted in findings of norepinephrine alone in the organ of Zuckerkandl. In man this organ disappears early in infancy, and it never seems to acquire the ability to methylate norepinephrine.

Recent studies by Hagen[14] showed that norepinephrine was formed from dopamine, and this finding has been supplemented by Neri *et al.*,[15] who found that if dopamine, adenosine triphosphate (ATP) and either diphospho- or triphosphopyridine nucleotide were incubated with bovine adrenal medulla, a norepinephrine-like substance was obtained. Cantoni,[16] investigating the general enzymatic mechanisms of methylation, found that the methyl donor was S-adenosyl-methionine, and that the enzymatic removal of phosphate groups from ATP is necessary; however, an "onium" compound like betaine can transfer one of its methyl groups without an energy source such as ATP.

If a metabolic block occurs in this chromaffin tissue, one would expect to find formation of intermediates at normal or reduced levels and the reduction or absence of later stage products. In a type of psychosis the metabolism of

L-tyrosine
↓ (oxidation)
L-dihidroxyphenylalanine (DOPA)
↓ (decarboxylation)
dopamine
↓ (oxidation)
norepinephrine
↓ (methylation)
epinephrine

could possibly be blocked at the methylation level. It would appear from clinical observations that this group of childhood schizophrenics, rather than exhibiting a metabolic block, retains the active chromaffin mass with its only function, the production of norepinephrine. However, a genetic factor could impair the enzymatic mechanism of transmethylation. Increased excretion of norepinephrine has been found in the urine of aggressive-hostile patients, and increased excretion of epinephrine in passive-fearful ones.[17]

The childhood schizophrenic presents varying degrees of language disorders (even mutism), compulsive behavior, withdrawal, lack of interpersonal relationships, disturbance in the appreciation and integration of identities and, in general, disorganization of behavior patterns that involve formation of contact with reality. Physical examination reveals dilation of pupils, hirsutism, primitive postural responses, and suppressed growth cycle. However, findings of hyperglycemia, hypertension, and hypermetabolism are not anticipated, since they occur only with the higher proportion of epinephrine to norepinephrine secreted by a chromaffinoma and adrenergic neurons. The latter condition is found usually in patients with pheochromocytoma and in one type of hypertension.

As has been pointed out previously,[9] it is my concept that amine oxidase inhibitors are highly specific for diseases of affect and that the tranquilizers are equally so for schizophrenia, even though the recovery with the latter therapy is certainly not as complete (about 20 per cent). Clinical exceptions thus far are that about 25 per cent of a type of schizophrenia respond favorably with amine oxidase inhibitors, and many agitated depressive patients respond favorably to reserpine therapy. The pharmacology of iproniazid is almost exactly the reverse of that produced by reserpine, with two notable exceptions: both reduce blood pressure and both act to prolong the action of barbiturates and other drugs. Reserpine may produce a depressive state, and iproniazid may produce exacerbation of an acute psychotic process, either manic with illusions or schizophrenic with delusions. These clinical observations would fit into the metabolic picture that depression, a disease of affect, is due to a reduction of norepinephrine, with a shift in ratio to other sympathomimetic amines. The schizophrenic process[9] is also the result of a shift in the sympathomimetic amines and their metabolic products of the transaminase system. I have found, in laboratory studies, that significant changes are obtained in nitrogen levels and amine metabolism[18] during the administration of phrenotropic drugs. I hope that further studies in sympathomimetic amine metabolism will increase our understanding of these diseases.

To be therapeutically effective in schizophrenic children (autistic) and in a type of adult psychosis characterized by aggressiveness, anxiety, hostility, compulsiveness, social detachment, and poor contact with reality, amine oxidase inhibitors must reduce the production of norepinephrine. It is conceivable from our present understanding of norepinephrine that this compound is largely, if not completely, formed from oxidation of dopamine. The oxidative deamination of dopamine, a better substrate for amine oxidase than norepinephrine and its metabolite, catechol acetic acid, is normally present in urine, according to Euler and Lishajko.[19] There is a conflict in published reports concerning changes in ratio of epinephrine to norepinephrine secretion upon electric or drug stimulation of the adrenals. Redgate and Gellhorn,[20] however, reported that electric stimulation of specific areas of the hypothalamus liberates from the adrenal medulla a variable percentage of epinephrine in the amine mixture. In these experiments what shifts, if any, in the percentage of methylation occurred? Are different sensory (afferent) impulses capable of shifting this ratio? This quantitative difference with the shift in ratio between norepinephrine and epinephrine may be due to the fact that there are probably two types of cells in chromaffin tissue. One type contains the enzymatic mechanism for methylation, but these cells may be insufficient in number to maintain the required physiological balance through methylation.

Epinephrine inhibits L-histidine decarboxylase, and this suggests that epinephrine may block the formation of histamine through a feedback mechanism. The role of histamine in the central nervous system remains unknown, but its localization indicates physiological significance in the limbic system. There are two histamine-metabolizing enzymes; besides histaminase (diamine oxidase), there is another system that is inhibited by iproniazid. This enzyme system, however, is composed of a methylating enzyme and an oxidative enzyme. Iproniazid blocks only the oxidative and not the methylating mechanism. Methylhistamine undergoes oxidative deamination from monoamine oxidase; therefore, the metabolism of histamine can be modified by levels of epinephrine and inhibitors of monoamine oxidase. This metabolic balance may be an essential factor in the psychic changes observed in depressed and schizophrenic patients under therapy with iproniazid.

Summary

Research, both *in vivo* and *in vitro*, into the role of amine oxidase and its inhibitors has demonstrated, in part, its function, adding to our knowledge of this enzyme system. These studies have also provided data elucidating the etiology and pathology of the following diseases: hypertensive cardiovascular complaints, angina pectoris, depression, schizophrenia (adult and childhood), and a group of miscellaneous clinical entities. There are many essential amines of the central nervous system, but the sympathomimetic amines of the chromaffin tissue and adrenergic neurons warrant intensified study. The lenticular nucleus, hypothalamus, and the pyramidal tracts were found to contain high amine oxidase activity and the following sympathomimetic amines: dopamine, norepinephrine, epinephrine, serotonin (possibly), methylhistamine, and others.

Histochemical evidence has been presented that demonstrates two types of cells in chromaffin tissue, one that contains mainly norepinephrine and the other epinephrine.[21] We may assume that the primary difference between the two cell types is that one has the enzymatic mechanism for methylating norepinephrine and the other does not. Therefore, if embryonic tissue such as the organ of Zuckerkandl remains active and adrenergic neurons in the central sympathomimetic areas are increasing the ratio of norepinephrine to epinephrine, a psychic pathology exists. These psychoses can be alleviated, in part, by amine oxidase inhibitors, reserpine, deserpidine, and some of the phenothiazines, probably by their ability to shift the norepinephrine-epinephrine ratio toward the physiological level. It is apparent from preliminary data that they accomplish this by different modes and at different sites of action. Fragmentary data indicate that this pathological state may be controlled by compounds that block oxidation of dopamine to norepinephrine, antagonize norepinephrine, substitute for epinephrine, and assist in methylation of norepinephrine. Data on these studies will be presented in the near future.

The amine oxidase inhibitor, iproniazid, has the ability to alleviate depressions by reducing the rate of oxidation and shifting the metabolic distribution in this disease of affect. By reducing excess norepinephrine production at the dopamine stage, it may be indicated in a type of schizophrenia. Current studies in our laboratories are directed toward elucidating these enzymatic mechanisms in sympathomimetic amine metabolism.[18]

Emphasis should be attached to the following areas:[22] enzymatic systems and mechanisms in formation, methylation, and oxidation of the sympathomimetic amines in the central nervous system; histochemical studies of the central nervous system in order to determine the sites and neurological systems involved in these disorders: depression, childhood schizophrenia, a type of adult schizophrenia, extrapyramidal syndromes (Parkinsonism), epilepsy, cardiovascular disease, and possibly others; and investigation of the possibility of an inherited deficit of an enzyme (methylpherase) necessary for the methylation of sympathomimetic and other amines.

References

1. Saunders, J. C. 1955. Commentary. Am. Psychiat. Assoc. Psychiatric Research Repts. No. 1: 152.
2. Saunders, J. C. & N. S. Kline. Drugs for treatment of depression. Neurology 9(4): 224.
3. Wilkins, R. W. 1954. Clinical usage of *Rauwolfia* alkaloids, including reserpine (Serpasil). Ann. N. Y. Acad. Sci. 59(1): 36.
4. Loomer, H. P., J. C. Saunders & N. S. Kline. 1957. A clinical and pharmacodynami evaluation of iproniazid as a psychic energizer. Am. Psychiat. Assoc. Psychiat Research Repts. No. 8: 129.
5. Saunders, J. C., D. Rochlin, N. Radinger & N. S. Kline. 1959. Iproniazid in depressed and regressed patients. *In* Psychopharmacology Frontiers. Chap. **32:** 177-194. Little, Brown. Boston, Mass.
6. Fallab, S. & H. Erlenmeyer. 1952. Zur Kenntnis des Isonikotinsaurehydrazid-Cu komplexes. Experentia. **8:** 298.
7. Saunders, J. C. 1957. Chelation—An integrative chemical concept of psychoses 2nd Intern. Psychiatric Conf. Zurich, Switzerland.
8. Zatman, L. J., N. O. Kaplan, S. P. Colowick & M. M. Ciotti. 1954. The isolation and properties of the isonicotinic acid hydrazine analogue of diphosphopyridine nucleotide. J. Biol. Chem. **209:** 467.
9. Saunders, J. C. 1959. Etiology of psychosis theorized from drug actions. *In* Psychopharmacology Frontiers. Chap. **64:** 407–412. Little, Brown. Boston, Mass.
10. Saunders, J. C. 1958. Discussion. J. Clin. Exptl. Psychopathol. **19**(2) suppl. 1: 84.
11. Bonvallet, M., P. Dell & G. Hiebel. 1954. Tonus sympathique et activité électrique corticale. Electroencephalog. and Clin. Neurophysiol. **6:** 119–144.
12. Holton, P. 1949. Noradrenaline in tumors of adrenal medulla. Am. J. Physiol. **108** 525–529.
13. Shepherd, D. M. & G. B. West. 1952. Noradrenaline and accessary chromaffin tissue Nature. **170:** 42–43.
14. Hagen, P. 1956. Biosynthesis of norepinephrine from 3,4-dihydroxyphenylethylamin (dopamine). J. Pharmacol. **116:** 26.
15. Neri, R., M. Hayano, D. Stone, R. I. Dorfman & F. Elmadjian. 1956. Conversio of hydroxytyramine to norepinephrine-like material. Arch. Biochem. Biophys. **60** 297–300.
16. Cantoni, G. L. 1957. Enzymatic mechanisms in transmethylation. *In* Metabolism of the Nervous System. : 481–492. Pergamon. London, England.

17. Elmadjian, F., J. M. Hope & E. T. Lamson. 1956. Excretion of epinephrine and norepinephrine in various emotional states. Federation Proc. **15:** 57.
18. Saunders, J. C., I. Lourie & H. Chipkiewicz. 1958. Biochemical studies in psychoses. 6th Bahamas Medical Conf. Nassau, B.W.I.
19. Von Euler, U. S. 1957. Adrenaline. *In* Metabolism of the Nervous System. : 543–552. Pergamon. London, England.
20. Redgate, E. S. & E. Gellhorn. 1953. Nature of sympathetico-adrenal discharge under conditions of excitation of central autonomic structures. Am. J. Physiol. **174:** 475–480.
21. Eränkö, O. 1955. Distribution of adrenaline and noradrenaline in adrenal medulla. Nature. **175:** 88–89.
22. Saunders, J. C. 1958. Some biochemical relationships of brain to psychopharmaceutical agents. Presented at Am. Psychiat. Assoc. Psychic Energizer Symposium. San Francisco, Calif.

THE INFLUENCE OF AMINE OXIDASE INHIBITORS ON EPINEPHRINE METABOLISM IN MAN*

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Zeller and his co-workers[1] have shown that iproniazid (1-isonicotinyl-2-isopropyl hydrazide, Marsilid†) is a very powerful inhibitor of monoamine oxidase, both *in vitro* and *in vivo.* Although iproniazid has also been found to inhibit other enzymes, the inhibition of monoamine oxidase has been the most studied. This is due to the fact that monamine oxidase has been found to be one of the enzymes involved in the metabolism of such potent biogenic amines as epinephrine, norepinephrine, serotonin, tyramine, and dopamine. When iproniazid was observed to counteract apathy and depression in mental patients, it was assumed by many that iproniazid acts by virtue of an inhibition of monoamine oxidase activity in the body. This concept was held despite the evidence that treatment with isoniazid (isonicotinic acid hydrazide, Rimifon†) produces a clinical response very similar to that produced by iproniazid,[2] and yet has little or no action on monoamine oxidase activity *in vitro.*[1]

In 1955 we undertook a study of the metabolism of epinephrine in man. Isotopically labeled epinephrine was infused into normal subjects and schizophrenic patients (FIGURE 1). When epinephrine labeled with carbon^{14} in the beta position of the side chain was infused, 91 ± 3 per cent of the administered radioactivity was excreted in the urine. When epinephrine labeled with carbon^{14} in the *N*-methyl group of the side chain was infused, 32 ± 2 per cent of the radioactivity was recovered in the urine (FIGURE 2). These data suggested that approximately two thirds of the molecules of infused epinephrine lose the *N*-methyl group during metabolism.[3, 4] Recent evidence seems to indicate that two major enzymes are responsible for the *in vivo* metabolism of catecholamines, namely, the *O*-methyl transferase system and monoamine oxidase.[5, 6] The radioactivity recovered in the urine following the infusion of beta-labeled epinephrine represents all of the metabolic transformations of epinephrine, since the beta carbon atom remains attached to the benzene ring. The radioactivity recovered in the urine following the infusion of methyl-labeled epinephrine (32 ± 2 per cent) represents the normal pool of nondegraded (free or conjugated) epinephrine, epinephrine acted upon by the *O*-methyl-transferase system and, possibly, a very small quantity of urinary products derived from methylamine.[7] When this pool is subtracted from 91 ± 3 per cent, the remainder represents those epinephrine metabolites that have been oxidatively deaminated by monoamine oxidase and have lost their radioactivity. Thus, the percentage of recovery of radioactivity in the urine following the infusion of methyl-labeled epinephrine is the only datum necessary from which one may assess the relative activity of the *O*-methyl-transferase system and the mono-

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† Hoffmann-La Roche.

amine oxidase involved in the *in vivo* transformations of infused epinephrine under any set of experimental conditions. Since monoamine oxidase is involved in the metabolism of the biogenic amines mentioned above, the data obtained following the infusion of methyl-labeled epinephrine should be an accurate method for the measurement of over-all monoamine oxidase activity in man.

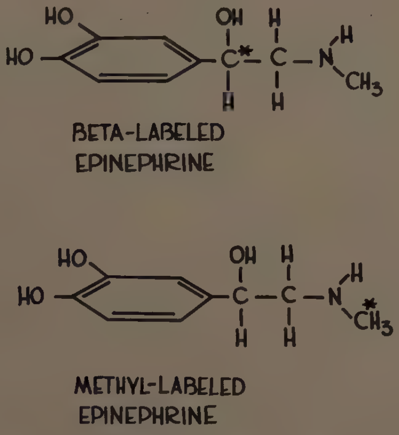

Figure 1. Epinephrine containing isotopic carbon.

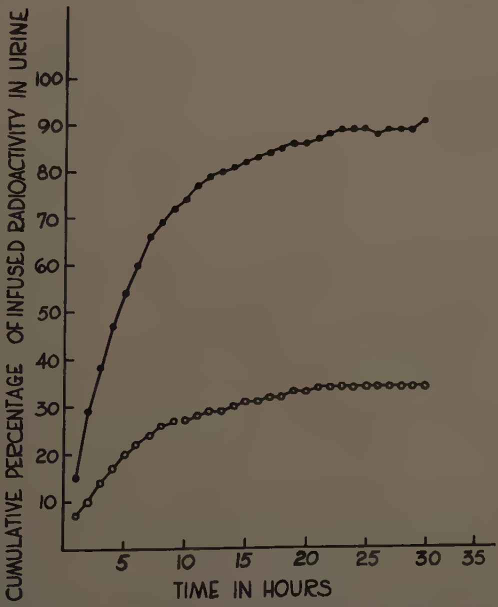

Figure 2. The average cumulative hourly urinary excretion of total radioactivity. A solid dot represents the average value for infusions with beta-labeled epinephrine; an open circle represents the average value for infusions with methyl-labeled epinephrine.

To test the validity of this hypothesis, psychotic patients were placed on iproniazid and infused with methyl-labeled epinephrine. The excretion of total radioactivity in the urine was determined and the urine selectively extracted for phenolic amines (metabolites due to *O*-methylation) and for phenolic acids (metabolites due to oxidative deamination).

The results obtained following infusion with methyl-labeled epinephrine are shown in TABLE 1. The excretion of total radioactivity in the urine of nontreated patients was 32 ± 2 per cent. In iproniazid-treated patients the total radioactivity excreted in the urine was 63 ± 3 per cent.[4] One patient was reinfused with methyl-labeled epinephrine 2 weeks after the cessation of iproniazid treatment, and 50 per cent of the radioactivity was recovered in the urine. Another patient was reinfused 3 weeks after cessation of iproniazid treatment, and 43 per cent of the infused radioactivity was recovered in the urine. Thus, approximately twice as many molecules of infused epinephrine retain the *N*-methyl group of the side chain when the patient is under iproniazid treatment in the dosages mentioned as when he is not. It can be seen that monoamine oxidase activity was inhibited by approximately 50 per cent at this dosage level. Approximately one half of the effect of iproniazid on monoamine oxidase activity was still evident 2 to 3 weeks after the cessation of iproniazid treatment.[4]

TABLE 1

URINARY EXCRETION OF RADIOACTIVITY FOLLOWING THE INFUSION OF 1 MG. (5.2 μC.) DL-EPINEPHRINE-METHYL-C^{14} INTO SCHIZOPHRENIC PATIENTS

Subject	Sex	Age	Length of treatment with Marsilid (100 to 150 mg./day)	Cumulative infused radioactivity excreted in the urine (per cent)
R. D.	Male	37		28
D. C.	Male	45		41
P. M.*	Female	50		32
D. C.	Male	45		33
F. L.	Male	46		25
C. B.	Male	65		33
J. K.	Male	68	10 days	55
F. K.	Male	34	24 days	64
M. C.	Female		11 weeks	59
M. S.	Female	61	13 weeks	74
J. H.	Female	31	16 weeks	63
M. C.	Female		Post-Marsilid 2 weeks	50
M. S.	Female	61	Post-Marsilid 3 weeks	43

* Bilaterally adrenalectomized.

It can be seen from the radioactivity data that approximately one third of the metabolites of infused epinephrine in nontreated patients are compounds with the *N*-methyl group intact. The remaining two thirds are presumably phenolic acids. After several weeks of treatment with iproniazid (100 to 150 mg./day) the proportion is reversed.

Friend and his collaborators[8] found no significant effect of iproniazid on cardiovascular activity or the plasma levels of norepinephrine infused into normal subjects. Resnick *et al.*[4] have demonstrated that iproniazid treatment in psychotic patients resulted in an accumulation of the urinary amine metabolites of infused epinephrine. Typical cardiovascular responses to infused epinephrine were also observed in the iproniazid-treated patients. Thus, it would appear that iproniazid treatment in man inhibits monoamine oxidase activity without potentiating the cardiovascular effects of the catecholamines and without increasing the peripheral levels of the catecholamines. Thus, with respect to cardiovascular action, the infused catecholamines are inactivated by a mechanism previous to monoamine oxidase action. This could be due either to the action of the *O*-methyl-transferase enzyme system or to a binding previous to enzymatic transformation.

Is the effect of iproniazid on behavior related to the inhibition of monoamine oxidase activity? An attempt to resolve this problem is now in progress. Three severely depressed psychotic females who had been receiving 150 mg. of iproniazid per day for 3 to 4 months were selected for a preliminary study. According to a personal communication from Harry Freeman, Medfield State Hospital, Medfield, Mass., 2 of the patients responded to iproniazid treatment, while the third showed no improvement. The patients were interviewed twice weekly and were rated by means of the Malamud-Sands rating scale.[9] These 3 patients were then infused with *N*-methyl-C^{14}-epinephrine. Fifty-nine, 63, and 74 per cent, respectively, of the infused epinephrine was recovered in the urine of these 3 patients. Hence, these patients showed a similar degree of monoamine oxidase inhibition, yet they varied markedly in their behavioral responses to iproniazid. Psychotic patients are now being treated with several iproniazid analogues. The psychiatric evaluations are being carried out by Harry Freeman and his staff. The effects of the iproniazid analogues on monoamine oxidase activity are being evaluated by means of infusion with *N*-methyl-C^{14}-epinephrine.

If the evidence should lead to the conclusion that there is no direct relationship between behavioral responses and monoamine oxidase inhibition, then three alternative mechanisms for the action of iproniazid and its analogues present themselves.

(1) All subjects, whether normal or psychotic, react to monoamine oxidase inhibition by an accumulation of those metabolites of biogenic amines formed previous to monoamine oxidase action. Certain nervous systems are capable of responding to an excess of such metabolites, while other nervous systems are not. In practice, it has been found that it is primarily the depressed and apathetic patient who responds to the psychic energizers. However, not all cases of depression respond to such therapy.

(2) In general, the central nervous system is not affected by the accumulation of such metabolites, although other systems of the body may be. This could explain the lack of correlation between the behavioral effects and the systemic effects observed in subjects treated with the psychic energizers.

(3) The systemic responses and the behavioral responses, when they occur, may be due to direct action of the psychic energizers themselves. If this should be the case, then the reactivity of the nervous system or more likely of the different parts of the nervous system may vary between normals and psychotics or between classes of normals and psychotics.

Preliminary investigations are now in progress in an attempt to elucidate some of the biological actions of metanephrine (MN), normetanephrine (NMN), and iproniazid and iproniazid analogues. The use of the isolated rat colon and estrous uterus for the bioassay of the catecholamines is well known.[10] The acetylcholine (Ach)-induced contractions of the isolated rat colon and estrous uterus can be inhibited by the addition of catecholamines to the incubation medium. This bioassay was employed to screen the actions of the catecholamines and their methoxy derivatives, iproniazid, and several of its analogues, several amine oxidase inhibitors that are not iproniazid analogues, the tranquilizing drugs, and 2 hallucinogens; the preliminary results are shown in TABLE 2. It was observed that MN, NMN, iproniazid, isoniazid, 1,2,3,4-tetrahydrophthalazine HCl (RO 5-0994) and 2-ethyl-benz[f]isoindoline hydrobromide citrate (RO 2-6797/5) potentiate, to a varying degree, the Ach-induced contractions in milligamma quantities. In microgram quantities MN, NMN, iproniazid, isoniazid, and RO 2-6797/5 inhibit, to a varying degree, the Ach-induced contractions. 2-Benzyl-1-picolinyl hydrazine (RO 5-0700) and 1-benzyl-2-(5-methyl-3-isoxazolyl carbonyl) hydrazine (RO 5-0831/1) markedly inhibit the Ach-induced contractions, both in milligamma and microgram quantities. However, after the inhibition is produced the succeeding Ach-induced contractions tend to become potentiated. From these very preliminary observations it can be seen that iproniazid and isoniazid demonstrated the same type of action on the isolated smooth muscle of the rat. This may be of significance, since it was cited above that both compounds produced similar clin-

TABLE 2

ACTIONS OF VARIOUS COMPOUNDS ON THE ISOLATED RAT COLON AND ESTROUS UTERUS MADE TO CONTRACT BY ACETYLCHOLINE*

Compound	Small doses	Large doses
Metanephrine (MN)	Potentiation	Inhibition
Normetanephrine (NMN)	Potentiation	Inhibition
Marsilid (iproniazid)	Potentiation	Inhibition
Rimifon (isoniazid†)	Potentiation	Inhibition
2-Ethyl-benz[f]isoindoline hydrobromide citrate (RO 2-6797/5)‡	Potentiation	Inhibition
1,2,3,4-Tetrahydrophthalazine HCl (RO 5-0994)‡	Potentiation	Potentiation
2-Benzyl-1-picolinylhydrazine (RO 5-0700)†	Inhibition	Inhibition
1-Benzyl-2-(5-methyl-3-isoxazolyl carbonyl) hydrazine (RO 5-0831/1)†	Inhibition	Inhibition
Mescaline	Potentiation	No Effect
Lysergic acid diethylamide (LSD-25)	Potentiation	Inhibition
Equanil (meprobamate)	Potentiation	Inhibition
Reserpine	Potentiation	Inhibition
Sparine	Inhibition	Inhibition
Phenergan	Inhibition	Inhibition
Epinephrine	Inhibition	Inhibition
Norepinephrine	Inhibition	Inhibition

* A modification of the Gaddum and Lembeck technique for bioassay of the catecholamines[10] was employed.

† Iproniazid analogue.

‡ An amine oxidase inhibitor that is not an analogue of iproniazid.

ical responses, whereas iproniazid inhibits monoamine oxidase activity and isoniazid does not.

It was also observed that MN and NMN demonstrated an action on the isolated smooth muscle of the rat similar to that of both isoniazid and iproniazid. Since it may be postulated that the concentration of MN and NMN increases with iproniazid, but not with isoniazid treatment, then iproniazid may be reinforced by MN and NMN in producing its effects on the receptor sites of the cells.

It is hoped that further work will help answer the question of the mechanism of action of the psychic energizers. It is also hoped that this work may result in the assignment of a physiological role to the metabolites of epinephrine and norepinephrine.

Acknowledgments

I am indebted to Fred Elmadjian, Harry Freeman, and Jane Wolfe, who were my collaborators in some of the studies discussed in this review. The work on psychotic patients was the result of cooperative research between the Worcester Foundation for Experimental Biology, Shrewsbury, Mass., and the Worcester State Hospital, Worcester, Mass. Several iproniazid analogues were generously supplied by Hoffmann-La Roche Inc.

References

1. Zeller, E. A., J. Barsky, J. R. Fouts, W. F. Kircheimer & L. S. Van Orden. 1952. Influence of isonicotinic acid hydrazide (INH) and 1-isonicotinyl-2-isopropyl hydrazide (IIH) on bacterial and mammalian enzymes. Experientia. **8:** 349.
2. Delay, J. & J. F. Buisson. 1958. Psychic action of isoniazid in the treatment of depressive states. J. Clin. Exptl. Psychopathol. & Quart. Rev. Psychiat. Neurol. **19**(2): Suppl. 1, 51.
3. Resnick, O. & F. Elmadjian. 1958. The metabolism of epinephrine containing isotopic carbon in man. J. Clin. Endocrinol. and Metabolism. **18:** 28.
4. Resnick, O., J. M. Wolfe, H. Freeman & F. Elmadjian. 1958. Iproniazid treatment and metabolism of labeled epinephrine in schizophrenics. Science. **127:** 1116.
5. Axelrod, J. 1957. O-Methylation of epinephrine and other catechols *in vitro* and *in vivo*. Science. **126:** 400.
6. Armstrong, M. D. & A. McMillan. 1957. Identification of a major urinary metabolite of norepinephrine. Federation Proc. **16:** 146.
7. Schayer, R. W., R. L. Smiley & E. H. Kaplan. 1952. The metabolism of epinephrine containing isotopic carbon II. J. Biol. Chem. **198:** 545.
8. Friend, D. G., M. S. Zileli, J. T. Hamlin, III. & F. W. Reutter. 1958. The effect of iproniazid on the inactivation of norepinephrine in the human. J. Clin. Exptl. Psychopathol. & Quart. Rev. Psychiat. Neurol. **19**(2): Suppl. 1, 61.
9. Ferreira, A. J. DeL. & H. Freeman. 1958. A clinical trial of Marsilid in psychotic depressed patients. Am. J. Psychiat. **114:** 933.
10. Gaddum, J. H. & F. Lembeck. 1949. The assay of substances from the adrenal medulla. Brit. J. Pharmacol. **4:** 401.

Discussion of the Paper

Yutaka Kobayashi (*Worcester Foundation for Experimental Biology, Worcester, Mass.*): Resnick and his group have published the first papers in the field of human metabolism of isotopically labeled epinephrine.[1, 2] Resnick's finding that epinephrine metabolism can be altered by treatment with iproniazid (Marsilid) is at present well established for both human and animal subjects[3-5] and needs no further discussion. However, an important observation that does deserve attention is the finding that in man the biochemical effects of iproniazid, as measured by the metabolism of methyl-labeled epinephrine, persisted over a period of 2 to 3 weeks after the cessation of the iproniazid treatment. This may be correlated with the report of Sidney Udenfriend elsewhere in this monograph that, in animals treated with iproniazid, monoamine oxidase activity, as measured by the metabolism of 5-hydroxytryptamine, was inhibited, and that approximately one week was required for complete recovery of normal enzyme activity.

In TABLE 1, Resnick's figures for human metabolism of isotopically labeled epinephrine are compared with those of the Axelrod group[6] at the National Institutes of Health, Bethesda, Md., and the Kirshner group[7] at Duke University, Durham, N. C.

Resnick, using C^{14} methyl-labeled epinephrine, recovered 32 per cent of the infused radioactivity in the urine. This fraction contained unchanged epinephrine, 3-methoxyepinephrine, conjugates of both amines, and methylamine metabolites. Resnick also infused β-labeled epinephrine and recovered 91 per cent of his radioactivity in the urine. It was calculated that 59 per cent of this radioactivity was probably due to acidic metabolites of epinephrine. The fact that Resnick recovered 91 per cent of the infused radioactivity with β-labeled epinephrine suggests that his analytical method was satisfactory. Resnick did not attempt any quantitation of the various epinephrine metabolites in the urine other than to determine unchanged epinephrine, which was found to range between 1 to 5 per cent. Resnick identified qualitatively the presence of the various metabolites of epinephrine using chromatography.

TABLE 1

HUMAN METABOLISM OF ISOTOPICALLY LABELED EPINEPHRINE

Laboratory	Type of isotopic epinephrine	Percentages of radioactivity present as:	
		Phenolic amines	Phenolic acids
Resnick *et al.*[1]	*N*-Methyl C^{14}	32	
	$\beta - C^{14}$		59
LaBrosse *et al.*[6]	$\beta - H^3$	54*	36 (VMA†)
Kirshner *et al.*[7, 8]	$\beta - C^{14}$	(1)4 to 6	67 to 85
		(2)0 to 51	39

* 3-Methoxyepinephrine, free and conjugated, only.

† 3-Methoxy-4-hydroxymandelic acid.

The Axelrod group,[6] using tritiated epinephrine, reported that 3-methoxyepinephrine and its conjugate account for 54 per cent and 3-methoxy-4-hydroxymandelic acid (VMA) for another 36 per cent of the radioactivity in the urine. Thus, this group can account for 90 per cent of the radioactivity in the urine in terms of these metabolites and demonstrates the sufficiency of their analytical methods.

The Kirshner group,[7] using β-labeled epinephrine, originally reported that only 4 to 5 per cent of radioactivity in the urine was due to phenolic amines, and that 67 to 85 per cent was due to phenolic acids. In a subsequent paper,[8] the figures were revised to 51 per cent phenolic amines and 39 per cent phenolic acids. This was a result of the finding that one of the original acidic components was a conjugate that could be hydrolyzed to yield free 3-methoxyepinephrine. Although the Kirshner group recovered only 76 per cent of the infused radioactivity in the urine, 95 per cent of the radioactivity in the urine was accounted for in terms of some metabolite of epinephrine, which again speaks well for their analytical methods.

Although the Kirshner and Axelrod groups are now essentially in agreement with each other, it is difficult to ascertain a flaw, if one exists, in Resnick's data or analytical methods. All 3 groups infused approximately the same amount of epinephrine into their respective subjects. The Axelrod group did their work with tritiated epinephrine, while the other two used carbon^{14} epinephrine. All three groups used β-labeled epinephrine, but Resnick also used methyl-labeled epinephrine. One interesting difference is that Resnick used schizophrenic patients, while the Axelrod and Kirshner groups used normal subjects. It is unlikely, however, that the metabolism of epinephrine by schizophrenics would differ markedly from normals, although this must remain a possibility.

Resnick's analytical method is by far the simplest in that it requires only an assay for the total amount of radioactivity excreted by individuals infused with methyl-labeled epinephrine. The recovered radioactivity must be a measure of all the *N*-methyl-containing metabolites of epinephrine produced and assigns a maximum value possible for any one metabolite in this fraction. The puzzling fact is that Resnick's total recovery of radioactivity is only 60 per cent of the value for 3-methoxyepinephrine, free and conjugated, found by both the Axelrod and Kirshner groups. All 3 laboratories are in fairly close agreement on their respective values for free epinephrine and free 3-methoxyepinephrine which account for 15 per cent or less of the amine fraction. Consequently, this apparent divergence must lie in the determination of conjugated 3-methoxyepinephrine. The disagreement among the laboratories is on a quantitative rather than a qualitative basis. Under the circumstances, a critical evaluation of each analytical method would be desirable.

With the discovery of more potent and specific inhibitors of monoamine oxidase such as those being considered in this monograph and the ever-increasing knowledge of the end products of epinephrine metabolism, the complete elucidation of the metabolism of epinephrine must be close at hand.

References

1. Resnick, O. & F. Elmadjian. 1958. The metabolism of epinephrine containing isotopic carbon in man. J. Clin. Endocrinol. and Metabolism. **18:** 28–35.
2. Resnick, O., J. M. Wolfe, H. Freeman & F. Elmadjian. 1958. Iproniazid treatment and metabolism of labeled epinephrine in schizophrenics. Science. **127:** 1116–1117.
3. Schayer, R. W. & R. L. Smiley. 1953. The metabolism of epinephrine containing isotopic carbon. III. J. Biol. Chem. **202:** 425–430.
4. Schayer, R. W., K. Y. T. Wu, R. L. Smiley & Y. Kobayashi. 1954. Studies on monoamine oxidase in intact animals. J. Biol. Chem. **210:** 259–267.
5. Schayer, R. W., R. L. Smiley, K. J. Davis & Y. Kobayashi. 1955. Role of monoamine oxidase in noradrenaline metabolism. Am. J. Physiol. **182:** 285–286.
6. LaBrosse, E. H., J. Axelrod & S. S. Kety. 1958. O-methylation, the principal route of metabolism of epinephrine in man. Science. **128:** 593–594.
7. Goodall, McC., L. Rosen & N. Kirshner. 1958. Catabolites of dl-adrenaline-2-C^{14} and effect thereon of Marsilid. Federation Proc. **17:** 56.
8. Kirshner, N., McC. Goodall & L. Rosen. 1958. Metabolism of dl-adrenaline-2-C^{14} in the human. Proc. Soc. Exptl. Biol. Med. **98:** 627–630.

CHEMICAL TREATMENT OF DEPRESSION

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The initial reports on the effectiveness of iproniazid for depressive states have spurred interest in this drug and other amine oxidase inhibitors. This report presents the clinical observations made during the treatment of 201 patients with iproniazid (Marsilid*), 50 patients given RO 4-1018 (the isopropylhydrazide of an aminomercaptobutyric acid), 25 patients treated with RO 2-6797 (2-ethyl-benz[f]isoindoline hydrobromide), and 20 patients administered RO 5-0700 (2-benzyl-1-picolinyl hydrazine). Included in this group were ambulatory and hospitalized, acute and chronic, neurotic and psychotic individuals chosen at random, not on the basis of their clinical diagnosis, but because depressed mood, psychomotor retardation, feelings of guilt, hopelessness, or despair, insomnia, anorexia, or functional somatic complaints were the predominant symptoms of their illness.

Clinically, each case was considered acute regardless of the duration of the illness because of the intensity of the affective disturbance and its effect on behavior. Since the majority of patients were ambulatory, information gathered from relatives and family physicians was collated with the impressions of the investigator. Each drug was administered without comment as to its identity or its expected clinical response. Likewise, to determine the effect of the drug alone, no other drug or any form of psychotherapy was given unless needed during the period of treatment.

Iproniazid

Iproniazid was prescribed for 61 men and 140 women (ages 10 to 79) diagnosed as follows: psychoneurosis; (depressed) 7; mixed, 28; obsessive-compulsive, 10; manic-depressive (depressed), 77; involutional depression, 54; and schizophrenia (paranoid), 5; unclassified, 14; and schizoaffective reaction, 6. The most severely depressed were given 50 mg. 2 or 3 times daily, the mild and moderately ill patients 10 or 25 mg. 3 or 4 times a day. The initial dose was continued until improvement occurred or toxic side effects intervened. Then the dosage was reduced gradually or discontinued if toxic side reactions persisted in spite of counteracting medication. Except for patients intolerant of iproniazid or those who refused to take it because of side effects, all patients received the drug for from 3 months to 1 year.

Because iproniazid is a slow-acting drug, therapeutic response seldom occurred before the second week of treatment; however, side effects were noted shortly after treatment began. Because of side reactions 43 patients (21 per cent) were unable to take iproniazid and had to be dropped from the investigation.

To assay the benefits of iproniazid therapy, the following criteria were employed: (1) marked improvement, that is, the total disappearance of depressive symptoms, and (2) moderate improvement, that is, partial modification of some or all of the target-depressive symptoms. By these standards 50 (31.6 per cent) of the 158 patients treated for 3 months to 1 year were markedly improved, and 71 (44.9 per cent) were moderately improved. However, of the 201 patients initially started on iproniazid only 24.8 per cent were markedly improved and 35.4 per cent moderately improved (TABLE 1).

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* Hoffmann-La Roche.

There was no apparent clinical difference between those who reacted well and those who did not improve, with one exception, namely, those patients previously unresponsive to electroconvulsive therapy. As a group, these shock-resistant patients responded best. Sex, age, and duration or severity of illness did not play a role in therapeutic results. Retrospectively, it is apparent that most of the improved patients experienced an onset of symptomatic relief within 2 weeks after the beginning of iproniazid, 75 to 150 mg./day, whereas the unimproved showed little or no change after 8 weeks of treatment. The majority of those patients with an endogenous depression who responded well to iproniazid had histories of previous depressions that warranted hospitalization or shock therapy.

TABLE 1

IPRONIAZID THERAPY: DIAGNOSTIC GROUPING, SEX DISTRIBUTION, AND THERAPEUTIC RESULT

Diagnosis	Male			Female		
	Marked	Moderate	Unimproved	Marked	Moderate	Unimproved
Psychoneurosis						
Depressed	1	1			1	4
Mixed	1	2	4	4	9	8
Obsessive-compulsive	1	2		1	1	5
Manic-depressive						
Depressed	7	10	11	14	15	20
Involutional depressions	5	5	3	12	16	13
Schizophrenic reactions						
Paranoid	1			1		3
Unclassified		2	2	1	3	6
Schizoaffective	1	1	1		3	
Total	17	23	21	33	48	59

Every patient had side reactions to iproniazid; some serious, others minor (TABLE 2). Somatic reactions occurred as follows: constipation, 56 per cent; dizziness, 46 per cent; dryness of the mouth, 50 per cent; weakness and fatigue, 39 per cent; headaches, 19 per cent; epigastric distress, 18 per cent; blurred vision, 27 per cent; itching, 14 per cent; sweating, 11 per cent; delayed micturition, 11 per cent; edema, 11 per cent; insomnia, 9 per cent; dyspnea, 7 per cent; neuralgic pains, 7 per cent; diarrhea, 7 per cent; paresthesia, 6 per cent; dermatitis, 4 per cent; impotence, 3 per cent; hyperreflexia/clonus, 3 per cent; anemia, 2 per cent; withdrawal symptoms, 1 per cent; hepatomegaly, 1 per cent; and cardiac failure, 1 per cent. Marked weight increases occurred which, both generally and individually, exceeded those usually seen with the recovery of depressed patients. In summary, 87.4 per cent of the patients with marked improvement and 79 per cent of the moderately improved showed progressive weight gains, while only 31 per cent of the unimproved gained weight. Conversely, 69 per cent of the failures did not gain or lose weight during the treatment.

The blood pressure response to iproniazid was variable and unpredictable. All patients with systolic pressures below 100 mm. Hg had no change or a rise in blood pressure. Of 151 normotensive patients (systolic pressure 100 to 150), 136 had no blood pressure change, 9 had an average rise of 30 mm. Hg (range 20 to 52), and 6 had an average decrease of 27 mm. Hg (range 20 to 50). Of 39 hypertensive patients (systolic 150+), 21 had no blood pressure alteration and 18 had an average drop of 35 mm. Hg (range 20 to 55).

TABLE 2
SIDE EFFECTS OF IPRONIAZID

Side effects	Male	Female
Postural hypotension	4	17
Edema	3	28
Paresthesias	1	11
Dyspnea	1	14
Cardiac failure	1	1
Headaches	9	30
Blurred vision	15	39
Dryness of mouth	30	70
Dizziness	24	68
Delayed micturition	7	15
Impotence	6	0
Constipation	28	85
Neuralgic pains	2	12
Hyperreflexia/clonus	2	4
Weakness and fatigue	18	60
Itching	8	22
Sweating	6	17
Diarrhea	5	10
Insomnia	4	15
Anemia	0	3
Epigastric distress	5	32
Dermatitis	1	7
Withdrawal symptoms	0	2
Hepatomegaly	1	1

Postural hypotension, a most dangerous side reaction of iproniazid, occurred in 11 per cent of the patients irrespective of their pretreatment blood pressure. These postural hypotensive episodes occurred suddenly and without warning. They were responsible for serious falls, with bodily injury. Several patients sustained extensive bruises and minor lacerations; 2 patients had severe facial and scalp lacerations; 2 others had hip fractures; 1 had a skull fracture; and another a fractured humerus. There was no correlation between the dosage of iproniazid and postural hypotension. Women had postural hypotension 4 times as often as men. In each instance the patient was older than 35.

Although some patients had edema, delayed micturition, and hepatomegaly while taking iproniazid, repeated urinalysis, blood counts, and liver function studies (cephalin flocculation, alkaline phosphatase, direct and indirect bilirubin, and thymol turbidity) did not disclose any significant deviation from the normal. Even the patients with hepatomegaly had normal liver function studies.

Iproniazid sensitizes patients to histamine release, barbiturates, novocaine, and anesthetic agents. One patient had a shocklike reaction to a wasp sting, with prostration, dyspnea, and cyanosis for which emergency hospitalization was required. Other patients reacted adversely to novocaine administered for dental work.

Psychic reactions to iproniazid were common. They were contingent upon the patient's pretreatment personality. Some patients were made more anxious, others more depressed, and still others euphoric and stimulated. Three patients with a prior history of manic and hypomanic reactions became hypomanic. Two patients had paranoid reactions with excitement; in these instances the drug unmasked an underlying psychopathological predisposition. Four patients developed a state of toxic psychokinetic stimulation that resembled an excitatory drug effect. The psychotic reactions developed abruptly between the sixth and eighth weeks of treatment on doses between 50 and 125 mg. daily. They were preceded by a brief spell of restlessness, enhanced anxiety, and insomnia. Psychomotor excitement, euphoria, and garrulousness were the characteristic symptoms of these psychoses. It was necessary to hospitalize all but 1 patient for treatment with tranquilizing drugs and electroconvulsive therapy. It took from 2 to 4 months for the patients to recover from their iproniazid-induced psychosis.

There was no positive correlation between dosage and the occurrence of side reactions. Some patients tolerated 150 mg. daily well, while others had multiple and, at times, serious reactions on not more than 75 mg. a day. The longer a patient took the drug, the more likely he was to have side effects, even when the dosage was as little as 30 mg. daily. Men had fewer side reactions than women; the older the patient, the more sensitive he was to the drug.

RO 4-1018

RO 4-1018 was prescribed for 16 men and 34 women (ages 18 to 74) diagnosed as follows: psychoneurosis (mixed), 1; reactive depression, 7; manic-depressive (depressed), 21; involutional depression, 17; paranoid depression, 1; schizoaffective reaction, 2; and senile depression, 1. Those patients with the most pronounced target symptoms received 50 mg. 2 or 3 times daily; the mild and moderately ill patients were given 25 mg. 2 or 3 times a day. The initial dose was continued until improvement occurred or toxic side effects intervened, when the dosage was reduced gradually or discontinued if toxic side reactions persisted in spite of counteracting medication. All patients received the drug for from 3 weeks to 6 months.

As with iproniazid, therapeutic response seldom occurred before the second week of treatment. However, side effects were noted shortly after treatment began. The same criteria for therapeutic results employed for iproniazid were used for RO 4-1018. By these standards 12 (24 per cent) were markedly improved, 18 (36 per cent) were moderately improved, and 20 (40 per cent) were unimproved (TABLE 3).

As with iproniazid, the best results were obtained in shock-resistant patients. Sex, age, and duration or severity of illness did not play a role in therapeutic outcome. Most of the improved patients manifested an onset of symptomatic relief within 2 weeks after the beginning of treatment, while the unimproved showed practically no change at the end of 2 months. Endogenously depressed patients with a history of prior depressions with hospitalization or shock therapy also responded favorably.

TABLE 3
RO 4-1018 THERAPY: DIAGNOSTIC GROUPING, SEX DISTRIBUTION, AND THERAPEUTIC RESULT

Diagnosis	Male			Female		
	Marked	Moderate	Unimproved	Marked	Moderate	Unimproved
Psychoneurosis						
Mixed		1				
Manic-depressive						
Depressed	1	3	3	5	4	5
Involutional depression	1	2	2	4	3	5
Reactive depression		1		1	3	2
Paranoid depression			1			
Senile depression						1
Schizophrenic reaction						
Schizoaffective reaction			1		1	
Total	2	7	7	10	11	13

TABLE 4
RO 4-1018: SIDE EFFECTS

Side effect	Male	Female
Weakness	5	11
Dizziness	7	20
Dryness of mouth	5	20
Constipation	6	15
Hypotension	4	
Sweating	2	16
Epigastric distress	5	14
Decreased erotic desire	4	1
Increased psychomotor activity	1	1
Edema of ankles	2	7
Hepatomegaly	0	1

Every patient had side reactions to RO 4-1018 (TABLE 4). These were: dizziness, 54 per cent; dry mouth, 50 per cent; constipation, 42 per cent; sweating, 36 per cent; epigastric distress, 34 per cent; weakness and fatigue, 32 per cent; edema, 18 per cent; postural hypotension, 15 per cent; decreased erotic desires, 10 per cent; increased psychomotor activity, 6 per cent; and hepatomegaly, 2 per cent. Marked weight increases that exceeded those usually seen with the recovery of depressed patients were common. Those patients who failed to gain more than 5 lb. or who lost weight did not benefit from RO 4-1018.

RO 4-1018 affected the blood pressure in the same manner as iproniazid. Although postural hypotension occurred, it was not as severe as with iproniazid, and no serious falls were reported.

Repeated urinalysis, blood counts, and liver function studies were within the range of normal variation. The liver function studies of the patients with enlarged livers were normal.

There was no positive correlation between dosage and the occurrence of side reactions. Some patients tolerated well 150 mg. daily, while others had multiple reactions on not more than 75 mg. daily. The longer a patient took the drug, the more likely he was to have side effects, even when the dosage was reduced to as little as 25 mg. daily. Men had fewer side reactions than women; the older the patient, the more sensitive he was to the drug.

RO 2-6797

RO 2-6797 was administered to 9 men and 16 women (ages 29 to 67) diagnosed as follows: reactive depression, 5; manic-depressive (depressed), 9; involutional depression, 9; and schizoaffective reaction, 2. Those patients with the most pronounced target symptoms took 50 mg. 2 or 3 times daily; the mild and moderately ill patients 25 mg. 2 or 3 times a day. The initial dose was continued until improvement occurred or toxic side effects intervened. Then the dosage was reduced gradually or discontinued if toxic side reactions persisted in spite of counteracting medication. All patients received the drug for from 2 weeks to 4 months.

Table 5
RO 2-6797 Therapy: Diagnostic Grouping, Sex Distribution, and Therapeutic Result

Diagnosis	Male			Female		
	Marked	Moderate	Unimproved	Marked	Moderate	Unimproved
Manic-depressive Depressed	1	1	1	1	2	3
Involutional depression	1	2	1	1	2	2
Reactive depression			2			3
Schizophrenic reaction Schizoaffective reaction						2
Total	2	3	4	2	4	10

As with iproniazid, therapeutic response seldom occurred before the second week of treatment. However, side effects were noted early. The same criteria for therapeutic results employed for iproniazid were used for RO 2-6797. By these standards, 4 (16 per cent) were markedly improved, 7 (28 per cent) were moderately improved, and 14 (56 per cent) were unimproved (TABLE 5).

The most favorable results were obtained in patients with an endogenous depression with a history of prior depressions, with hospitalization or shock therapy. Most of the improved patients showed an early response to the drug, whereas the unimproved ones experienced no change at the end of 2 months.

The side reactions to RO 2-6797 were: anorexia, 72 per cent; weakness, 56 per cent; constipation, 48 per cent; dry mouth, 32 per cent; insomnia, 32 per cent; decreased erotic desires, 32 per cent; dizziness, 24 per cent; nausea, 24 per cent; and increased psychomotor activity, 24 per cent (TABLE 6). Repeated urinalyses, blood counts, and liver function studies were normal.

Unlike the other amine oxidase inhibitors, RO 2-6797 was an appetite depressant. Its administration caused weight loss in 75 per cent of the patients, the remaining 25 per cent gained no more than 5 pounds. There was no correlation between weight fluctuation and therapeutic outcome. Also, unlike iproniazid and its analogues, RO 2-6797 had no effect on blood pressure. The clinical effects of RO 2-6797 were in many respects similar to those produced by amphetamine.

RO 5-0700

RO 5-0700 was given to 5 men and 15 women (ages 29 to 70) diagnosed as follows: psychoneurosis (mixed), 2; obsessive-compulsive, 1; reactive depression, 3; manic-depressive (depressed), 6; involutional depression, 5; senile depression, 1; and schizoaffective reaction, 2. The most depressed patients were given 10 mg. 3 times daily; the mild and moderately ill patients 10 mg. twice daily. The initial dose was continued until improvement occurred or toxic side effects intervened. Then the dosage was reduced gradually or discontinued if toxic side reactions persisted in spite of counteracting medication. All patients received the drug for from 10 days to 3 months.

TABLE 6
RO 2-6797: SIDE EFFECTS

Side effect	Male	Female
Weakness	7	7
Dizziness	1	5
Dryness of mouth	1	7
Constipation	3	9
Erotic desire (decreased)	3	5
Nausea	3	3
Increased psychomotor activity	1	5
Anorexia	8	10
Insomnia	3	5

As with the other drugs, therapeutic response seldom occurred before the second week of treatment, and side effects were noted early. The same criteria for therapeutic results employed for iproniazid were used for RO 5-0700. By these standards, 3 (15 per cent) were markedly improved, 5 (25 per cent) were moderately improved, and 12 (60 per cent) were unimproved (TABLE 7).

The most favorable results were obtained in patients with an endogenous depression with a history of prior depressions, with hospitalization or shock therapy. Most of the improved patients showed an early response to the drug, whereas the unimproved experienced no change at the end of 2 months.

The side reactions to RO 5-0700 were: epigastric distress and nausea, 60 per cent; weakness and fatigue, 45 per cent; insomnia, 45 per cent; dizziness, 35 per cent; constipation, 35 per cent; weight gain, 35 per cent; increased psychomotor activity, 30 per cent; diarrhea, 25 per cent; headache, 20 per cent; and edema, 20 per cent (TABLE 8). Generally these side effects were milder than similar ones produced by the other amine oxidase inhibitors. Repeated blood counts, urinalyses, and liver function studies were normal. Several patients had some lowering of blood pressures (average 15 mm. Hg), but there were no instances of postural hypotension. There was no positive correlation between dosage and the occurrence of side reactions. Men had fewer side reactions than women; the older the patient the more sensitive he was to the drug.

TABLE 7
RO 5-0700 THERAPY: DIAGNOSTIC GROUPING, SEX DISTRIBUTION, AND THERAPEUTIC RESULT

Diagnosis	Male			Female		
	Marked	Moderate	Unimproved	Marked	Moderate	Unimproved
Psychoneurosis						
Mixed						2
Obsessive compulsive						1
Manic-depressive		1	1	1	1	2
Depressed						
Involutional depression	1	1		1	2	
Reactive depression			1			2
Senile depression						1
Schizophrenic reaction						
Schizoaffective reaction						2
Total	1	2	2	2	3	10

TABLE 8
RO 5-0700: SIDE EFFECTS

Side effects	Male	Female
Epigastric distress/nausea	4	8
Headache	0	4
Dizziness	1	6
Constipation	2	5
Diarrhea	1	4
Weakness and fatigue	3	6
Insomnia	2	7
Increased psychomotor activity	2	4
Weight gain	2	5
Edema	1	3

Comment

The amine oxidase inhibitors can be potent stimulants that produce a sense of well-being, increase energy, abolish fatigue, lessen the need for sleep, affect sexual desire, make a person loquacious, and may produce a psychosis. Hence, they may have amphetaminelike action. These drugs also can cause weakness and fatigue, drowsiness, and postural hypotension. In this respect they resemble tranquilizers. They also may be psychotomimetic drugs, especially when prescribed for patients with a previous psychotic episode or a predisposition to a psychosis. For these reasons these drugs should be prescribed cautiously for ambulatory patients who cannot be carefully supervised. Their use should be restricted to the severely ill patient.

Three pertinent observations were made in this survey: (1) patients who have side effects such as headaches, dizziness, diarrhea, anorexia, and weight loss shortly after therapy is started do not benefit from these drugs; (2) patients who have not responded within two months are not likely to improve with further therapy; and (3) it is essential to know the patient's physical and mental condition and basic personality structure before prescribing an amine oxidase inhibitor.

That amine oxidase inhibitors may benefit depressed patients cannot be denied. However, their usefulness is limited by side effects, some of which are potentially serious. Admittedly, most side effects can be managed by counteracting drugs such as sedatives, tranquilizers, vitamins, hormones, steroids, and diuretics, which lessen the risk of this type of chemotherapy. On the other hand, not a few patients need these drugs for prolonged periods even to obtain partial modification of depressive symptoms. That these drugs affect many catecholamines is evident from their clinical effects. What adverse effects prolonged administration of amine oxidase inhibitors will have on the liver is not yet known. Even though the patients in this study who have enlarged livers do not have abnormal liver function tests, we cannot assume the absence of hepatic pathology resulting from these drugs. More time is needed to assess their safety, as well as their true clinical value. In the interim we should look for more effective and safer amine oxidase inhibitors for the chemical treatment of depression.

Discussion of the Paper

CARL BREITNER (*Arizona State Hospital, Phoenix, Ariz.*): The trend of development in psychiatric research is largely indicated by conferences such as that upon which this monograph is based and that held in Rome, Italy, in September 1958.[1] Obviously, the chemists are in the limelight, but chemists usually are not trained in psychiatry, and psychiatrists are not trained in chemistry. Therefore, a link is needed between the two disciplines, and such a link is provided by investigators such as Frank Ayd.

Publications by physicians on the use of new drugs were once considered of minor importance and were often regarded with suspicion as being subsidized by the drug companies. With the correlation of chemistry and psychiatry becoming essential for further progress, this picture has changed. Clearly, we are in the midst of an important phase of development of knowledge relating to the etiology of mental illness. Since etiology has, or should have, a bearing on therapy, I should like to discuss etiology.

When Sigmund Freud discovered the etiological significance of the unconscious, many psychiatrists thought that the major etiological factors of mental disorders had been discovered. As a result, where psychiatrists used the term dynamics, they inferred that these dynamics were merely psychological. In fact, it might be said that the great discoveries of Freud hindered in some respects the complete understanding of etiology. Thus mental illness, including the major psychoses, was subjected to analytical interpretation, and other possible contributing factors were pushed in the background. Those physicians who used somatic methods of treatment were often belittled, and mental illness was conveniently divided into organic and functional disorders. According to this classification, "functional" includes what, by present means, cannot be seen *in vivo* or post-mortem, and therefore is considered as psychological.

Only in the last five years has the psychiatric world begun to pay attention to the development of functional illness in patients with at least concomitant organic disorders, namely, those in senility, post-traumatic psychoses, post-partum psychoses, involutional psychoses (including surgical menopause syndromes), and in the neuroses of menstruating women.

According to analytical concepts, people suffered from psychotic depressions because of some trauma in early childhood or infancy. It was thought that it sometimes took twenty to forty years to develop the symptoms of illness. Today, at least the possibility of a reversal of the cause-and-effect relationship is considered, and we find published opinions to the effect that depression causes guilt feelings over past events, rather than that guilt feelings cause the depression.[2]

The study of affective psychoses, the ones most susceptible to our modern methods of treatment, may well lead to better understanding of the etiology of other psychoses, and it may not be too optimistic to think that a single organic disorder of chemical origin may be at the root of all psychotic behavior. To elucidate this possibility: less than one year ago I met with skepticism when I casually mentioned that catatonic schizophrenia and depression were the same illness, but of different degrees. This possibility led me to try iproniazid (Marsilid) in stuporous catatonics, the results indicating that both categories respond to the same treatment, namely: electroconvulsive therapy (ECT), as well as Marsilid.[3]

Further, in reviewing the literature, I found that several textbooks[4, 5] described severe depression exactly as we describe a catatonic schizophrenia, namely: symptoms of waxy flexibility and regression, with mutism, inability to eat, and untidiness. Therefore, catatonia and depression must be similar in appearance as they are similar in their response to treatment. However, the catatonic obviously has features in common with other schizophrenics; in other words, depression resembles catatonia, and since catatonia is schizophrenia there may well be one common cause for depression and schizophrenia.

To go one step further, there are indications that paranoia and depression may have one and the same origin, namely: a disorder in the primitive brain, while the cortex—or call it the personality structure—determines whether the patient will turn his hostility toward himself and become depressed or toward the environment, becoming paranoid.

In fact, one entity in our diagnostic classification—the involutional psychosis, which may lead to either paranoid or depressive manifestations—is supposed to originate at least partly in a chemical disorder. Perhaps it is optimistic to think that we are not far from being able to work out a new classification of mental illness based on etiology rather than on symptomatology.

As to Ayd's paper, it is one of many contributions that he has made in the field of correlation between psychiatry and biochemistry. Ayd discussed some new compounds that are in the experimental stage and with which I have had no experience. However, I should like to make a few comments concerning Marsilid, which we have used for about 18 months at the Arizona State Hospital (a study of this drug has been reported elsewhere).[6] We have found Marsilid a very useful drug in all types of depression, particularly in cases resistant to electroconvulsive therapy. We usually begin with 50 mg. 3 times daily, the dosage being reduced as the patient develops hyperreflexia. Using the drug in more than 100 patients, we have not encountered any serious side effects, which is at variance with Ayd's report of side effects in 100 per cent of his cases. The explanation of this discrepancy may well lie in the fact that Ayd included among side effects such symptoms as weakness and fatigue, headaches, epigastric distress, and insomnia. These symptoms may well not be side effects of the drug but perhaps should be classified as symptoms of the disease.

Among the advantages of the drug is the fact that it increases appetite; since Marsilid has been available, we have not had a single case where tube feeding of patients had become necessary. Another important point is that Marsilid has helped patients who previously had been treated, without lasting results with many other means, including ECT. This fact may not show up statistically, but the individual patient and his family are not as much interested in statistics as in the results in the particular individual. If some patients who had been unable to leave the hospital and remained sick in spite of all other treatments have recovered and have been returned to society, obviously an attempt to use the drug should be made where other means have failed. It is to be hoped that the newer drugs may be even more effective and less dangerous to use. However, in my opinion, Marsilid is no more dangerous than the phenothiazines, which are generally acknowledged to be helpful and are used liberally.

Ayd is an objective investigator, and if he states that he prefers some newer compound to Marsilid, I am inclined to deduce that these are even better. He is to be congratulated on his work, which I believe is of great benefit to our profession.

References

1. First International Congress of Neuro-Psycho-Pharmacology. Rome, Italy. Sept. 8 to 13, 1958.
2. Kraines, S. H. 1957. Mental Depressions and Their Treatment. Macmillan. New York, N. Y.
3. Breitner, C. 1958. Marsilid in catatonic schizophrenia. Am. J. Psychol. **114**(10): 931.
4. Noyes, A. 1955. Modern Clinical Psychiatry. 3rd ed. : 334–339. Williams & Wilkins, Baltimore, Md.
5. Henderson, D. K. & R. D. Gillespie. 1940. A Textbook of Psychiatry. 5th ed. : 147–156. Oxford University Press. New York, N. Y.
6. Breitner, C. 1958. Iproniazid in the Treatment of Mental Disorder Resistive to Previous Therapy. *Read at* Symposium on Monoamine Oxidase Inhibitors. San Francisco, Calif. Sept. 25.

THE USE OF IPRONIAZID IN THE TREATMENT OF DEPRESSIVE STATES

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Robert Burton, the celebrated Oxford clergyman, published his famous *Anatomy of Melancholy* in 1621. Among other things, this work is a scholarly catalogue of therapeutic efforts against depressive states from the time of Hippocrates and Democritus to that of his own Seventeenth Century England. Burton regarded the many-faceted disorder melancholy as both a spiritual and bodily infirmity. More than 250 years later we find ourselves thinking in the same general fashion about the etiology of these disorders of mood and equally avid in our quest for effective methods of treatment.

Iproniazid (1-isonicotinyl-2-isopropyl hydrazine), Marsilid,* synthesized for the first time in 1951, is one of the latest in the long list of medicinal agents employed in this long search for a successful treatment for these mysterious disturbances of mood. This report deals with our clinical study of this compound during the past two years.

Method

The compound was dispensed from the office and/or hospital. In most instances the patients were unaware of the name, chemical nature, or anticipated action of the drug, and were not advised of the side effects they might expect. No formal double-blind or placebo studies were conducted. The drug was administered orally.

Selection of Cases

Private patients with a variety of neuropsychiatric conditions were treated with varying doses of iproniazid. The compound was administered orally, total daily dosage varying from 25 to 200 mg./day. The usual dosage range was 50 to 150 mg./day, given in divided doses.

In our study iproniazid has now been used in the treatment of approximately 500 patients. Our findings in the first 268 seen during the first year of the drug's use are analyzed here. It is our feeling that similar results have accrued from the larger series, even though the last 225 cases have not been tabulated at this writing. The group included both office and hospitalized patients suffering from neurotic, psychotic, and/or neurological disorders. Most of the patients had depressive symptoms either as a primary or complicating factor in their illness. They varied in age from 10 to 78 years.

Results

In all instances a delay in the onset of action was apparent and varied from 3 days to 6 weeks. The majority of the patients showed an initial response in 1 to 3 weeks. Dosage adjustment was prompted by failure of response or the appearance of side effects. If the patient failed to respond within approximately 2 weeks the dosage was increased. If the side effects occurred, the initial dosage was decreased. After an optimum dosage was reached, this level was maintained for 8 to 10 weeks and then gradually reduced. Determination of duration of maximum effective dosage was difficult; if symptoms recurred, the dosage was temporarily increased to the previous effective level and reduced again later. Early in the study the maximum dosage was continued for a longer period of time than in the more recent cases. The level of dosage could not be correlated with the severity of the illness or the incidence or severity of side effects.

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* Hoffmann-La Roche.

Table 1

Types of Cases Included in the Psychotic Group

Psychotic reactions	
Psychotic depressive reactions	69
Manic-depressive, depressive phase	16
Schizoaffective	11
Post-partum psychosis	10
Involutional psychotic depression	22
Schizophrenic reactions	3
Neurotic reactions	
Neurotic depressive reactions	76
Obsessive-compulsive personality	7
Post-traumatic neurosis	7
Neurasthenia	2
Alcoholism	2
Mixed psychoneurosis	19

Table 2

Diagnostic Categories of 24 Depressive Patients

Organic and neurological disorders with depressive reactions	
Multiple sclerosis	1
Parkinson's disease	2
Postleukotomy state	2
Cerebral thrombosis with hemiparesis	2
Neurodermatitis	1
Chronic organic brain syndrome	3
Narcolepsy and cataplexy	2
Epilepsy	4
Huntington's chorea	1
Thalamic syndrome	1
Speech tics and impediments	2
Coronary disease with anginal pain	1
Labyrinthitis	2

Of this group of 268 patients (61 males and 207 females) approximately 50 per cent of the patients were psychotic, 40 per cent were neurotic, and 10 per cent had neurological disorders. The breakdown of the group is shown in TABLE 1.

An additional 24 patients suffered from depressive symptoms associated with other organic neurological syndromes and chronic diseases. Their various diagnostic categories are given in TABLE 2.

Results were rated as excellent, good, fair, and failure. An excellent result was one in which the patient gained complete relief from his chief complaints and was able to attain his previous level of social and occupational adjustment, a good result was characterized by definite improvement in the patient's symptoms and social adjustment, and a fair result was one in which the patient gained only minimal and partial relief from his symptoms (TABLE 3).

TABLE 3

RESULTS OF IPRONIAZID THERAPY ON 268 PATIENTS

	No.	Excellent	Good	Fair	Failure
Psychotic					
Psychotic depressive reaction	69	36 (52%)	15 (22%)	2 (3%)	16 (23%)
Manic-depressive (depressive state)	16	5 (31%)	4 (24%)	0	7 (44%)
Schizoaffective	11	1 (9%)	2 (18%)	0	8 (73%)
Post-partum	10	2 (20%)	7 (70%)	1 (10%)	0
Schizophrenia, simple	3	1 (33⅓%)	1 (33%)	0	1 (33⅓%)
Involutional depression	22	9 (40%)	5 (23%)	3 (14%)	5 (23%)
Neurotic					
Neurotic depressive reaction	76	20 (25%)	24 (31%)	8 (10%)	24 (34%)
Obsessive-compulsive	7	2 (28%)	3 (43%)	0	2 (29%)
Post-traumatic	7	0	1 (14%)	3 (43%)	3 (43%)
Neurasthenia	2	0	0	0	2 (100%)
Alcoholism	2	0	1 (50%)	0	1 (50%)
Mixed psychoneurosis	19	1 (5%)	8 (42%)	3 (16%)	7 (37%)
Organic and neurological disorders with depressive reactions					
Multiple sclerosis	1		1		
Parkinson's disease	3	1	1		1
Postleukotomy state	2			1	1
Cerebral thrombosis with hemiparesis	2			2	
Neurodermatitis	1		1		
Chronic organic brain syndrome	3	1		1	1
Narcolepsy and cataplexy	2	1			1
Epilepsy	4	1		2	1
Huntington's chorea	1		1		
Thalamic syndrome	1				1
Speech tics and impediments	2		1	1	
Coronary disease with anginal pain	1		1		
Labyrinthitis	2		1		1

Electrocoma therapy was employed in the psychotic reactions when failure of response or severe side effects necessitated discontinuing the compound.

Side effects were present in 125 patients (47 per cent). The more frequent side effects are shown in TABLE 4.

Of the 125 patients showing side effects, it was necessary to withdraw the drug from 33 patients. Although side effects were frequent and sometimes distressing, most patients elected to continue the medication because of the improvement it had produced. Occasionally it was necessary to insist that the drug be discontinued. In some patients side effects could be controlled by reduction of dosage.

Comment

This drug has been fascinating to us. The specificity of its action in depressive states is something new in our clinical experience. Pharmacologically, it appears to act differently from the analeptics, such as the amphetamines and the piperidyls, which have been used heretofore in the treatment of depressions.

Our clinical experience with iproniazid has posed many questions. If one achieves a good therapeutic result, when is the right time to withdraw the drug? We have seen no evidence of addiction to it, but patients are sometimes reluctant to stop it, especially if we suggest that this be done soon after improvement occurs. On occasion we have stood by passively and allowed the patient to follow his own choice in this regard. If this is done, the patient usually begins to "forget" to take a portion of his medication in the second month after improvement occurs, and stops it completely in about 3 to 4 months, on the average. This, allowing for individual variation, has now become our usual practice.

Table 4

Side Effects of Iproniazid in 125 of 268 Patients (47 per cent)

Type	No. cases	Percentages of total patients
Constipation	36	22.5
Hypotension	27	16.5
Vertigo	17	10.2
Hyperreflexia	15	9.0
Clonus	12	7.2
Syncope	12	7.2
Impotence	10	6.0
Edema	9	5.4
Neuralgia	4	2.4
Tension	5	3.1
Excitement	4	2.4
Psychosis	2	1.2
Jaundice	3	1.8
Delay in micturition	3	1.8
Insomnia, severe	2	1.2
Nausea and vomiting	1	0.6
Weakness	1	0.6

It is interesting to contrast the subjective reaction of the depressive patient to electroconvulsive (ECT) treatment versus Marsilid treatment in successful cases. In our experience, the patient treated electrically seldom gives the treatment technique much credit for producing his recovery; he is inclined to look back on the illness and say that he "pulled himself together" and "snapped himself out of his illness." With Marsilid he is more likely to give the drug a good deal of credit for his improvement. This is especially true if a dramatic improvement begins to take place within 7 to 10 days after the drug is started. Although no hard-and-fast rule can be given, it is our general impression that patients treated successfully with Marsilid are grateful: grateful for the drug and to the physician who prescribed it. This is sometimes the case with ECT treatment, to be sure, but is less common and less enthusiastically expressed in our experience.

One of the fascinating side reactions of iproniazid is the loss of libido it may produce on occasion; this may be as profound in the female as in the male. A young female hospital technician developed a disturbing situational depression over difficulties in adjusting to her new marriage. Her mood improved promptly after the administration of 25 mg. Marsilid twice daily, and she found herself able to cope with and solve many of her marital problems more easily thereafter. About 6 weeks later, however, she found herself incapable of orgasm and became completely indifferent to sexual activity. Previously this had been an important part of her marital relationship, and it had been one of the strong forces that had held the marriage together. She stopped taking Marsilid forthwith, and her quite active sexual life with her husband re-established itself within 2 weeks.

Are there any other drugs that may produce such a response? Perhaps, but none come quickly to mind. The whole problem of impotence and frigidity has been a complex one for the physician as long as he has been ministering to the sick. Causes are undoubtedly multiple, theories are many, and therapies are legion, but they are usually not very effective. Is there a biochemical element in some cases of sexual inertia? This thought has been expressed often, and has led to many types of hormonal, vitamin, and similar therapies. Marsilid poses a very real challenge in this regard. If the biochemical dynamics of the side reaction of impotence that this drug occasionally produces could be defined, it is reasonable to hope that a rational therapy for a portion of the cases of sexual impotence might be forthcoming by correcting such a biochemical error.

Anyone who has practiced psychiatry in the same comunity for a reasonable number of years acquires a large collection of recurrent depressive patients, and they teach many profound lessons. Although they differ from one to the other, their attacks may be quite regular and often follow a predictable pattern. They provide a test situation for any therapeutic regimen that can be more revealing than a statistical study. More than any other, this group demonstrates the therapeutic uses and limitation of Marsilid.

A 74-year-old refrigeration and air-conditioning engineer was inherited by our office some years ago after he had outlived 2 psychiatrists who had treated him in his earlier years. He began to have recurrent depressive attacks in his twenties; they appeared in approximately 3-year cycles and lasted for about 6 months. The attacks were divided into 3 equal parts: during the first 2 months he became progressively more involved with insomnia, anorexia, clouding of the sensorium, and psychomotor retardation. During the middle 2 months he was incapable of work, was moderately agitated, underwent a 30-pound weight loss, and had suicidal urges that never materialized in overt attempts on his life. The last 2 months were spent in convalescence. During the next 30 months he was a highly successful engineer, but he insisted on seeing his doctor at intervals during his periods of normal health in the hope that he could be kept out of a recurrence.

During the last decade his attacks could be brought to a halt by electroconvulsive treatment. Approximately 1½ years ago another attack descended upon him. His internist advised against electrotherapy because of coronary insufficiency, a slow fibrillation, and early myocardial weakness. Because his attacks were known to have a predictable pattern, it was decided to weather it through. In the third month his entire family formed a phalanx and descended upon our office with a demand that something be done about him. Everyone in his family circle was emotionally depleted by his illness. Marsilid (50 mg. twice daily) had been prescribed 5 days previously, but no change had occurred. The conference ended with the agreement that, if no change for the better had occurred within 1 week, he would be hospitalized.

A week later he walked into the office, obviously better. He apologized for being late, stating that he had been down to his office that morning and had become so engrossed in work that had piled up that he failed to look at his watch. He said, "You and the other doctors I've had have given me a carload of pills in my time, but none like this little fellow. Why didn't you use it long ago?" His son telephoned a few days later to report that the family were agreed that the change in his father had been profound. It is difficult to ascribe this change to anything but Marsilid. According to his demonstrated predictability pattern, he was supposed to remain ill for 3 more months. This patient has asked to remain on 25 mg. of the drug daily in the hope that it may have prophylactic value against an attack that may come later. He tolerates the drug well and says that he is as intellectually alert and as keen about his work as he ever was. We are continuing to allow him to use it. Are we correct in this?

In contrast, a fine little grandmother, aged 76, has had 14 documented depressive attacks during her adult life. Although she has little of this world's goods, she is a happy person when she is well, and her 6 children and her troop of grandchildren are devoted to her. Her attacks literally descend on her, and she inverts from her normal sunny state to that of whimpering befuddled depression within a week. She responds to 4 to 6 electroconvulsive treatments. When her fifteenth depression began some months ago, one of her sons brought her to us without delay. Marsilid (25 mg., first twice daily, then 3 times daily) was given without benefit. The dose was doubled and she began to have syncopal attacks. It was dropped back, but still no benefit occurred. ECT was given again, and her response was prompt.

Two other recurrent depressions may almost be considered a matched pair. An attorney who was experiencing his fifth depression began to respond to Marsilid 100 mg. daily on the third day after it was begun. He was fully restored in mood, intellectual efficiency, and physiological homeostasis within one week; in previous attacks he responded slowly and laboriously to ECT. A financier, similar in body build and cultural background to the attorney and only slightly older, failed to respond completely to 100 and then to 150 mg. Marsilid daily after 8 weeks of its use and went through his usual 5-month period of depressive turmoil until he recovered.

It appears obvious to us, as it does to many students of this drug, that it is dramatically effective in some depressive patients, while being totally ineffective in others. Other patients fall in the ground between the two extremes. They improve, but not dramatically and less fully. Most statistics reveal that it is of value in about two thirds of the cases. Why should such a potent therapeutic tool prove powerless in one third or more of the patients who receive it? There is a possibility that biochemical individuality in human beings may make the difference. The metabolic fate of isoniazid (isonicotinic acid hydrazide or INH) has been studied by Hughes and her associates.[1, 2] These investigators were concerned about the development of peripheral neuritis as a complication in about one third of tuberculosis patients who received this drug in large quantities. Since all the metabolites of INH are recoverable in the urine, it is possible to study the metabolic fate of this drug accurately.

Three distinct patterns of metabolic degradation of the drug were found. When acetylation of the terminal nitrogen of the hydrazine moiety or cleavage of the hydrazine to form isonicotinic acid occurred, no peripheral neuritis resulted. When difficulty in acetylation was present, and a third ill-defined pathway of degradation occurred, peripheral neuritis resulted. About one third of humans metabolize INH in each of these ways. Marsilid is the isopropyl derivative of INH, and a similar disturbance of metabolic degradation of this drug may be present in some people; these may fail to achieve any therapeutic benefit from it. At least, such an approach to a study of the exasperating difference in response to the drug might prove profitable. Clinicians would welcome analogues of this drug that would be equally as good as Marsilid, but more consistently so in depressive states.

Conclusions and Summary

Our analysis of this compound in the treatment of neuropsychiatric disorders was conducted on 268 patients over a period of 1 year. Since that time we have employed it in another 225 cases; although tabulation of these additional cases is not complete, it is our impression that the trend in results has not changed.

Iproniazid appeared to be more effective in the treatment of the psychotic reactions than in the neurotic disorders. The best results were obtained in the psychotic depressive reactions, in which a 77 per cent improvement rate was noted. The other psychotic reactions also showed good results, with the exception of the schizoaffective disorders.

In the neurotic group the drug was most effective in the neurotic depressive reactions and the obsessive-compulsive personality disorders. The neurasthenic and post-traumatic neuroses showed the poorest response to treatment.

It is interesting to note that beneficial effects were obtained in 66 per cent of the schizophrenic group who manifested depressive symptoms.

In the neurological disorders there was no evidence that the basic organic pathology was altered or aggravated. Partial or complete relief from depressive symptoms was obtained in 66 per cent of patients.

Since a large number of the psychotic depressive reactions was helped by the compound, the necessity for ECT was undoubtedly decreased; however, there are many cases in which the severity of the illness and/or the risk of suicide require immediate electroconvulsive therapy either alone or in conjunction with the administration of iproniazid.

No definite conclusions can be drawn concerning the time of appearance of side effects, but hypotension and constipation were generally noted earlier in the treatment. Hyperreflexia, clonus, impotence, and edema usually were later in appearance. Syncope was usually associated with postural hypotension.

Appetite remained good or increased in practically all patients, even though these showed other symptoms of autonomic nervous system involvement. The administration of B-complex vitamins, including pyridoxine, appears to aid in alleviating some side effects.

In the cases of jaundice, complete medical work-up revealed a clinical and laboratory picture typical of that seen in acute hepatitis. In our cases the jaundice cleared in two to three weeks, with cessation of the drug and general medical management. In one case the medication was again started in small doses with no recurrence of jaundice or liver disease, nor has any yet been noted. No fatalities have been noted in our series.

Even though iproniazid is considered to be a psychic energizer, it is interesting to note that there was a low incidence of disturbing side effects of overstimulation such as tension, insomnia, excitement, and psychotic confusion. We feel that the compound should not be used in cases with increased psychomotor activity.

We feel that here we have a drug which has a direct therapeutic effect, but an inconsistent one, on the depressive state.

References

1. Hughes, H. B. 1953. On the metabolic fate of isoniazid. J. Pharmacol. Exptl. Therap. **109:** 444.
2. Hughes, H. B. *et al.* 1954. Metabolism of isoniazid in man as related to the occurrence of peripheral neuritis. Am. Rev. Tuberc. **70:** 266.

Discussion of the Paper

Zale A. Yanof (*Toledo, Ohio*): With their customary efficiency, Fabing and his co-workers have presented a keen and critical study of a new drug. My experience with iproniazid (Marsilid) has been limited chiefly to the mild and moderate nonpsychotic depressives of general medicine. As with Fabing and his group, I soon found that the Marsilid effect was one that I had never seen clinically before. It is a potent psychic energizer and remarkable mood normalizer but quite different in its actions from the sympathomimetic amphetamine and allied compounds as racemic amphetamine sulfate (Benzedrine) and dextro-amphetamine sulfate (Dexedrine) and the newer ones, methylphenidylacetate hydrochloride (Ritalin) and 2-diethylaminoethyl benzilite (Benactyzine). While the latter drugs excite inhibited mental and emotional functions and bring about an undesirable euphoria, Marsilid seems to produce a cheerful and optimistic mood that approaches the normal state in the adjusted and contented individual. Undoubtedly, Marsilid is the nearest approach to the ideal mood elevator and restorer yet found.

In internal medicine, I have found Marsilid to be of great value in mild depression and its allied states of psychomotor retardation, including psychogenic fatigue, hypoenergization, moodiness, physical and mental inertia, reduced zest and vitality, and absence of optimism in thinking and feeling. Marsilid can also be used with great advantage in such diverse medical conditions as convalescent asthenia, postcoronary depression, rheumatoid arthritis, the common mild-to-moderate depression of older people, and other organic states with secondary depression. For these conditions, doses as low as 10 to 25 mg. every other day usually suffice.

I have had the same experience as Fabing *et al.* with patients who have responded well to Marsilid; they actually refuse to stop taking the drug. When Marsilid produces a positive response, the effect is so pronounced that adjunctive psychotherapy is rarely necessary. In my nonpsychotic group, the effect is usually seen by the third day, and rarely later than the sixth day, in contrast to the delay in action (as long as 6 weeks) reported by Fabing. Also, in contrast, I have encountered heightened sexual desire and tension much more frequently than impotence, but this may be due to the smaller doses in my series. Another interesting difference is that, while Fabing *et al.* report a low incidence of overstimulation (heightened tension, psychomotor excitement, and insomnia), I found overstimulation to be the most frequent side effect. This can usually be controlled by regulation and reduction of the dose. An early and reliable clinical sign of overstimulation is hyperreflexia.

In 150 cases, there was one instance of jaundice, which later was conclusively shown to be infectious mononucleosis, with liver involvement. All Marsilid-treated cases that develop jaundice should be carefully studied for infectious mononucleosis, with the thought in mind that the heterophil agglutination test may not become positive until the fourth or fifth week. Likewise, the abnormal lymphocytes of infectious mononucleosis may not appear until late in the disease. There is no definitive way of distinguishing infectious hepatitis from Marsilid-induced hepatitis. Even the liver biopsy is identical in both conditions, which poses a formidable problem in the distinction of the two. If both are followed from the beginning, however, the transaminase value in Marsilid hepatitis will rarely go above 400 to 600 units, while values of 1200 to 2000 units are common in infectious hepatitis.

In spite of its alleged or presumed hepatotoxicity, Marsilid can be safely used in the lesser depressions of general medicine if certain precautions are observed. First, it should be established through careful liver-function studies that there is no liver impairment. Weekly or biweekly enzymatic transaminase determinations then serve as a safe and positive guide to beginning liver dysfunction. The transaminase value, as recently developed by Wroblewski,[1-3] is a highly sensitive index of early liver damage and will be elevated as early as two weeks before any of the other liver function tests become positive.

Acknowledgment

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References

1. Wroblewski, F. 1959. Increasing clinical significance of alterations in enzymes of body fluids. Ann. Internal Med. **50:** 62–93.
2. Wroblewski, F. 1957. The significance of alterations in serum transaminases in hepatitis. *In* Hepatitis Frontiers. F. W. Hartman, G. A. Lo Grippo, J. G. Mateer & J. Barron, Eds. **:** 447–465. Little, Brown. Boston, Mass.
3. Wroblewski, F. 1957. The significance of alterations in serum enzymes in the differential diagnosis of jaundice. A.M.A. Arch. Internal Med. **100:** 635–641.

THE USEFULNESS OF AMINE OXIDASE INHIBITORS IN PSYCHIATRY

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As scientific knowledge progresses, accumulating evidence often allows the integration of two previously unrelated bodies of knowledge. Thus, new hypothetical constructs may facilitate further knowledge. Even at the risk of being overspeculative, efforts should be made continually to relate and integrate such knowledge. It seems that, with the present rapid advances in neurochemistry, a continual effort must be made to understand them in terms of psychology or behavioral science which, in essence, is the study of interpersonal relations or social behavior. Psychiatry is a pragmatic clinical science that requires a continual struggle at the crossroads of biological and social science and the adoption of what is helpful from both approaches. No system of evaluating behavior can escape the need for evaluating the cultures or social setting in which it occurs. No study of enzymes or chemistry can attain these objectives; that is, no enzyme or metabolic process can be expected to reveal the social consequences of its activity.

We shall present briefly an effort to integrate behavior theory with neurochemical hypothesis. In our opinion, the study of amine oxidase inhibitors may have its greatest usefulness in the development of theoretical concepts rather than in a direct therapeutic attack on artificial diagnostic categories of pathological social adjustment.

Several decades ago, in an effort to develop a motivational psychology, Sigmund Freud devised a technique of exploring the meaning behind apparently meaningless behavior or communication. This technique of psychoanalysis has been a valuable tool in explaining human behavior. As a result, the concept of unconscious motivation or unconscious mind is now almost universally accepted as valid in explaining human behavior. Its usefulness as a tool in investigation and treatment gradually led to the acceptance, without critical re-examination, of the idea that the unconscious mind is a department of the mind, a thing in itself, a place, or a concrete object.

It seems to us that re-examination of this concept is in order. To think of the unconscious mind as a functional system rather than a thing is in keeping with modern neurological theory of functional rather than topographical brain systems. We hope to present evidence of an approaching era when metabolic biochemical differences between this functional system (primitive emotive) and the higher levels of consciousness (cortical systems) can be demonstrated. The cortical systems, which serve a mediating, integrated, verbally communicative function in organizing adaptive social behavior, in a sense screen from us, the activity of the unconscious functions. They require that inferential processes be used to understand the unconscious function. Rado[1] described this phenomenon as follows:

"Freud used 'latent,' 'hidden,' and other cognate adjectives as equivalent to 'unconscious.' We suggest 'non-reporting' be added to this list. Terms qualified by any one of these adjectives are extrapolated psychologic terms. Speaking for instance, of an *unconscious* or *non-reporting desire*, the investigator refers to a missing causal agent, which . . . acted as if it had been a *desire*, though in fact it was a purely physiologic event. Since the psychologic meaning of non-reporting nervous activity can be arrived at only by this process of psychologic inference, naturally it can be expressed only in an extrapolated language of psychology."

MacLean[2] spoke of the same phenomenon in neurophysiological terms. He quoted the theory of Papez as follows: ". . . the hypothalamus, the anterior thalamic nuclei, the gyrus cinguli, the hippocampus and their connections constitute a harmonious mechansim which may elaborate the functions of central emotion, as well as participate in emotional expression." MacLean also cited evidence that the orbitomesial surface of the frontal lobes, the anterior insula, the temporal pole, and the pyriform-amygdaloid complex should be added to this system and referred to as the "visceral brain."

In primitive forms the visceral brain provides the highest correlation center for ordering the affective behavior of the animal in such basic drives as obtaining and assimilating food, reproducing, and fleeing from or orally disposing of an enemy. In human beings, visceral feelings are fused and blended with what the individual sees, hears, or otherwise senses in such a way that the world is often experienced or dealt with as if it were incorporated. The symbolizing, integrating, and remembering activities of the conscious cortical system bind time so that past experience, as well as present perception, influences this blending and fusing of internal and external perceptions. As MacLean[2] said: "Considered in the light of Freudian psychology, the visceral brain would have many of the attributes of the unconscious id. One might argue, however, *that the visceral brain is not at all unconscious . . . but rather eludes the grasp of the intellect (conscious awareness) because its animalistic and primitive structure makes it impossible to communicate in verbal terms*." Furthermore, the activities of this functional system, when viewed from the psychological standpoint, are altogether emotional and rudimentary. This mode of thought tends to identify with one or another rationally unrelated things, namely, confusion of internal and external perceptions. Much of the data is speculative and empirical when related to human behavior, although its neurochemical and neurophysiological attributes may be quite accurate. We can say at present only that evidence is accumulating that there is a metabolic system in the brain that coincides reasonably closely with a functional neurophysiological system and a psychoanalytical behavioral system.

Serotonin in brain tissue is normally present largely in bound form, and is thus protected from the action of monoamine oxidase. The amine has an asymmetrical distribution. The higher concentrations are found in the brain stem, that is, in subcortical areas and in relatively low concentrations in the cerebral cortex and cerebellum; the region of the hypothalamus is therefore one of higher concentration. This does not imply that the site of greatest drug concentration is necessarily the site of major action, but a study of the dynamics of drug distribution in the brain contributes to the concept of a chemical and metabolic, as well as anatomic and physiological, topography in the central nervous system. The studies with carbon^{14}-labeled amines have disproved the one-to-one relationship between vascularity and site of drug action.[3] There is a possibility that this functional system can be affected by drugs and other chemicals, but as yet we do not have sufficient knowledge to predict with any degree of certainty the effect on total human behavior. Even in those instances where it is predictable it is not explainable in the human being.

Recent demonstrations of Olds and Olds[4] have been published regarding these proposed transmitters and their breakdown in the brain by monoamine oxidase, which is inhibited by iproniazid (Marsilid*). Iproniazid may have its effect in augmenting the action of one or more of the following drugs: epinephrine, norepinephrine, and serotonin, which are concentrated in the hypothalamus and tegmentum and which exert known motivational function. Drugs that affect mental states also affect concentrations of these substances. Olds and Olds injected microgram amounts of chemicals into the hypothalamus of the rat to test for possible rewarding effects in areas where electric stimulation is highly rewarding. By these experiments iproniazid in quantities of 1 to 2 μg. is shown to be a rewarding stimulant. The rewarding effect of this chemical agent is specific to the injection site in the brain, a rewarding function that iproniazid appears to share with epinephrine. In these experiments animals were allowed self-injection of small quantities of iproniazid by bar pressing. In substance, Olds and Olds demonstrated that iproniazid has a reward-exciting function in the motivational system of the hypothalamus, and that it probably has this excitatory function in common with epinephrine. Thus, the relatively high concentrations of serotonin and, consequently, increased inhibitory activity of the hypothalamic and other limbic structures suggest the hypothesis that emotions mediated in these basic subcortical structures are alterable by pharmacological agents.

These animal experiments are extremely stimulating and provocative, but they offer little understanding of their significance in humans. The brains of animals are predominantly primitive; animal behavior is not modified by a thinking, symbolizing, remembering, rational cortical system such as characterizes the human. For this reason transposition of results of animal experimentation to human behavior is invalid, especially in predicting the effect of drugs on human behavior in which "goodness" or "badness" is culturally determined. The notion that human behavior is a result of the dynamic equilibrium between two functional systems in the brain—one a primitive, emotive, and instinctual system, and the other a rational, remembering, symbolizing, and synthesizing function—has been pointed out above. This concept has been elaborated and tested by behavioral scientists and clinicians in repeated applications to experimental situations. Neuroanatomic as well as neurophysiological evidence seems to support such a construct. Chemistry also is beginning to provide evidence to support this idea. Each new facet of evidence tends to substantiate the general theory, while at the same time requiring its refinement.

It appears that serotonin occurs in relatively greater concentrations in those systems referred to as the visceral brain. There is considerable evidence that these chemical manipulations can affect human behavior, thought, and mood.

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* Hoffmann-La Roche.

However, evidence has been presented also by one of us (R.L.F.)[5] that *Rauwolfia* compounds produce antitherapeutic results in some patients whose past experiences have affected the conscious remembering systems.

We are now presented with biochemical tools and knowledge that will enable us to modify the dynamic equilibrium between unconscious and conscious systems. This is a new and important lever, but its application requires detailed investigation of how past experience and personality and psychosocial adjustment modify its effects. It seems to us that the only way to carry out this investigation in the intact human being is by psychological inference as described by Rado:[1] through psychoanalytical and psychological techniques applied to human beings who are receiving drugs. Because of some doctrinal adherence to theories of behavior, this type of research has been delayed, preventing psychoanalysts and pharmacologists from working together on the same phenomenon exhibited on different levels of organization. In our opinion, however, such research must continue if we are to find a way to apply the available knowledge to cure or reduce mental illness.

References

1. Rado, S. 1949. Mind, unconscious mind, and brain. Psychosomat. Med. **11:** 165.
2. MacLean, P. D. 1949. Psychosomatic disease and the "visceral brain." Psychosomat. Med. **11:** 338.
3. Barlow, C. F., J. C. Schoolar & L. J. Roth. 1957. Distribution of carbon-14 labeled isoniazid in brain. Neurology. **7:** 820.
4. Olds, J. & M. E. Olds. 1958. Positive reinforcement produced by stimulating hypothalamus with iproniazid and other compounds. Science. **127:** 1175.
5. Faucett, R. L., E. M. Litin & R. W. P. Achor. 1957. Neuropharmacologic action of Rauwolfia compounds and its psychodynamic implications. A.M.A. Arch. Neurol. Psychiat. **77:** 513.

Discussion of the Paper

Sidney Malitz (*New York State Psychiatric Institute, New York N. Y.*): For me, the varied and sometimes contradictory results obtained by several of our distinguished colleagues have underscored the importance of the appeal implicit in the thoughtful and provocative paper of Frazier and Faucett. The very disagreements in these carefully evaluated findings emphasize the tremendous complexity of the behavior pattern of the total human organism. Unfortunately, this pattern is never as predictable or constant as some of its component parts when studied at the synaptic or biochemical substrate level.

The marked differences in drug action observed, not only from patient to patient but in any one patient at different times, must be related to other variables in the total adaptive pattern of the organism. Any theory that does not take these into account will embody handicapping scotomata that may lead to loss of sight of the forest for the trees. Results obtained with these drugs depend, not only on the reaction at the synapse and biochemical substrate level, but also at the interpersonal and environmental levels, including the doctor-patient relationship, the circumstances under which the drug is administered, the meaning of the drug to the patient in psychological terms, the personality structure of the patient, and his established patterns of adaptation to life and the life situation at the time he is given the drug. To assess these drugs properly, we must develop a system of equating and integrating these factors.

Of necessity, the tools used in this evaluation must draw heavily on the contributions of psychodynamics and psychoanalysis. While Sigmund Freud's contributions to the latter are undisputed, it is understandable that many of our colleagues in the basic sciences have been dissatisfied with his topographic metapsychology, and at times have even ridiculed it. This area is probably the weakest part of Freud's theoretical framework, and it may even have impeded the progress of the psychoanalytic method as a research tool. Fortunately, the number of psychiatrists who today accept this metapsychology as evidence of literal anatomic landmarks in the brain is steadily diminishing.

At present, behavioral alterations associated with the amine oxidase inhibitors appear to be quantitative rather than qualitative. There is still no drug that can in any way be called curative in the sense that it directly alters the causative factors in the most disturbing mental and emotional diseases, such as manic-depressive psychosis or schizophrenia. One of the chief reasons for this is that we still do not know what these factors are. That some of the amine oxidase inhibitors alter affectivity, however, now seems fairly convincing. While the basic pattern of adaption is not altered by these drugs, it now appears possible for a patient who has been frozen by his depression to mobilize his personality resources by means of these drugs so as to function in a more effective and self-gratifying fashion.

Great excitement has been stirred up by the brilliant work of many chemists, neurophysiologists, and pharmacologists concerning the role of serotonin and the catecholamines in mental illness. Frazier and Faucett have cited the remarkable work of Olds and Olds,[1] to which could be added the pioneer work of Woolley and Shaw,[2] Gaddum,[3] and Marrazzi,[4] and the interesting findings of Brodie and his group.[5] While alterations of these substances in the brains of mentally disturbed patients certainly occur, and while the amine oxidase inhibitors influence their levels, there is still no conclusive evidence that serotonin and the catecholamines are causally involved. In other words, these substances may be indicators of another more deep-seated and fundamental process, just as elevation or depression of body temperature may be an indication of any underlying bacterial infection.

I should like to underscore the authors' comments about basing conclusions on the actions of these drugs in experimental animals. As has been clearly demonstrated, some lower species of animals do not even possess the new parts of the brain in which the final synthesis of impulses from more primitive areas finally occurs in man. Therefore, the testing of these new drugs eventually must be performed in man.

The authors have mentioned doctrinaire differences that have impeded the amalgam of psychoanalytic and basic-science theory and techniques. To this might be added the semantic problem, just as in the political sphere our relations with certain foreign powers might be more compatible if more of us spoke one another's language. By extrapolating this philosophical concept, this would seem to apply to psychopharmacology.

Until the advent of the new psychopharmacological agents, psychiatrists and basic scientists spoke different languages with little inclination to learn each others' tongues. What we need now are still more translators, the interdisciplinary men who speak both languages with relative fluency. Such people are still hard to come by, but the advent of tranquilizers and amine oxidase inhibitors has increased their number. Through their efforts we will probably reach the goal for which Frazier and Faucett yearn: namely, the integration of neurophysiological, metabolic, and behavioral theories in understanding and influencing disordered human behavior.

References

1. OLDS, J. & M. E. OLDS. 1958. Science. **127:** 1175–1176.
2. WOOLLEY, D. W. & E. SHAW. 1954. Science. **119:** 587.
3. GADDUM, J. H. & K. A. HAMEED. 1954. Brit. J. Pharmacol. **9:** 240.
4. MARRAZZI, A. S. & E. R. HART. 1955. Science. **121:** 365.
5. BRODIE, B. B., J. S. OLIN, R. G. KUNTZMAN & P. A. SHORE. 1957. Science. **125:** 1293–1294.

CLINICAL EVALUATION OF PSYCHOSTIMULANTS

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In reviewing the psychiatric side effects of iproniazid (Marsilid), Crane[1] observed that the earliest reports did not stress psychiatric changes in response to this drug. However, in his series of 19 patients treated with iproniazid he found that psychic abnormalities predominated. Wiedorn and Ervin[2] reported 5 cases of schizophrenialike psychotic reactions associated with the administration of isoniazid or iproniazid in combination with other antituberculous drugs in the treatment of pulmonary tuberculosis. Kline[3] and Loomer *et al.*[4] administered iproniazid to a group of 20 female patients with chronic depressions or signs of withdrawal, of whom 18 exhibited some psychiatric changes; in 70 per cent of these cases the changes were considered to be favorable in nature. Freymuth *et al.*[5] studied the effects of iproniazid on a group of 54 chronically regressed schizophrenic patients. Using a double-blind technique, they included a control group, and in the iproniazid-treated patients they observed improvement in alertness, contact, and spontaneity as well as a small, but significant, number of reactivations of psychotic patterns. From these and other reports it may be inferred that iproniazid is a psychostimulant that produces changes in the areas of affect, motor activity, and social behavior of patients who have been hospitalized for prolonged periods. The purpose of this preliminary study was to determine whether screening of the comparative merits of 2 psychostimulants can be accomplished in a medium-size series of patients observed over a relatively short period of time. The compounds included in the program were iproniazid and one of its analogues, RO 4-1027, which chemically is 1-(*p*-chlorobenzoyl)-2-isopropylhydrazine. The study was conducted in the Continued Treatment Service of the New Jersey State Hospital at Trenton.

Case Material

The plan was to include 36 chronically depressed patients to be selected on the basis of their recorded diagnosis. Cases with clinically evident heart disease, renal impairment, or liver disease were not to be admitted to the program. However, on reviewing the case material of this 2000-bed hospital section, only 16 patients with chronic depression could be mustered. Consequently, it was decided to include, in addition, 20 chronic schizophrenic patients who exhibited symptoms of motor and psychomotor retardation and of social withdrawal, which they had in common with the depressed patients in the former group.

Before they were included in the present study, all patients were screened on the basis of ratings by the medical and nursing staffs, review of patients' records, screening interviews, Symptom Profiles,* and Wittenborn Psychiatric

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* The Symptom Profile, which contains 119 items of behavioral evaluation, arranged in 11 categories that correlate with the part reactions of our standard mental status examination, is used to obtain a tally of the occurrence and frequency of certain behavioral and symptom trends.

Rating Scales.[6] The entire series was divided into 2 major groups roughly matched on the basis of similar symptomatology: Group 1 exhibited primarily affective depression associated with changes in psychomotor and motor behavior, while Group 2 exhibited similar behavioral changes without affective depression.

Group 1. There were 16 patients in this group: 10 women and 6 men. Their ages ranged from 46 to 69 years, with a median age of 57 years. The total duration of hospital stay of these patients ranged from 0.5 to 40 years, with a median duration of 21 years. The diagnosis of all patients was manic-depressive, psychotic reaction: depressed type. The initial clinical profile included as main features moderate to severe depression, mild to severe motor and psychomotor retardation, mild to severe withdrawal from social, recreational, and occupational activities within the hospital, mild anxiety, and feelings of self-deprecation. All patients expressed suicidal ruminations, and 10 had made suicidal attempts. Eight patients experienced periodic phases of excitement, and 6 others exhibited clear-cut schizoid features. One patient later admitted having dissimulated a previous hallucinatory experience.

Group 2. There were 20 patients in this group: 18 men and 2 women. Their ages ranged from 22 to 64 years, with a median age of 51 years. The total duration of hospital stay of these patients ranged from 2 to 41 years, with a median duration of 22.5 years. The diagnosis of all patients was schizophrenic reaction classified according to the following types: hebephrenic in 7, catatonic in 5, undifferentiated in 5, simple in 2, and paranoid in 1. The initial clinical profile included as principal features apathy, autism, moderate to severe motor and psychomotor retardation, occasional secondary phenomenon, and moderate to severe withdrawal from social, recreational, and occupational activities within the hospital. No patient exhibited a depressed or anxious affective response.

METHOD OF STUDY

All patients had been treated previously with one or more psychopharmacological agents. This medication, if still given, was discontinued, so that all patients, at the time they were included in the present study, had been without such treatment for a period of 3 to 4 weeks. All male patients were maintained on one ward and all female patients on another. The study period extended over 50 days, or 10 phases of 5 days each, as follows: a 5-day pretreatment period of observation (first phase), a 40-day treatment period (second through ninth phases), and a 5-day post-treatment period of observation (tenth phase).

Fifteen patients, 6 in Group 1 and 9 in Group 2, were given iproniazid; and 21 patients, 10 in Group 1 and 11 in Group 2, received RO 4-1027 (see TABLE 1). The dosage schedule was the same for both medications, as follows: 75 mg./day for 5 days, followed by 150 mg./day for 30 days; this was reduced to 75 mg./day for the remaining 5 days of the treatment period. Medication was given 3 times daily throughout. The plan was to decrease dosage or interrupt treatment if major untoward effects occurred.

Psychiatric evaluations, determinations of physiological functions, and laboratory tests were made as described below.

During the base-line observation period (first phase) the following procedures were carried out: (1) a psychiatric evaluation that included the completion of a mental status examination, of the Wittenborn Psychiatric Rating Scale, and of the Symptom Profile; (2) daily interviews limited to specific questions concerning appetite, sleep, bowel habits, fatigue, and interest in ward activities and to recording of spontaneous complaints; (3) daily determinations of body temperature, pulse rate, respiratory rate, and supine as well as orthostatic blood pressures and a single determination of weight; and (4) laboratory tests, including total leukocyte and erythrocyte counts, differential counts, hemoglobin determinations, urinalyses, cephalin flocculation tests, and direct and indirect van den Bergh's tests.

During the treatment period (second through ninth phases) the following procedures were carried out: (1) daily evaluation of changes in degree of affective depression, motor and psychomotor retardation and social withdrawal, and completion of the Wittenborn Psychiatric Rating Scale and the Symptom Profile midway in the treatment period; (2) interviews as indicated above; (3) determinations of body temperature and so on, as outlined in the third procedure cited above; (4) laboratory tests as listed above at 8-day intervals, that is, 5 times during the treatment period; and (5) daily recordings of any untoward reactions possibly attributable to the medications.

TABLE 1
DIAGNOSTIC GROUPS OF 15 PATIENTS TREATED WITH IPRONIAZID AND 21 PATIENTS TREATED WITH RO 4-1027

Diagnosis	Total number of patients	Number of patients receiving	
		Iproniazid	RO 4-1027
Group 1			
Manic-depressive, psychotic reaction: depressed type	16	6	10
Group 2			
Schizophrenic reaction	20	9	11
Totals	36	15	21

During the post-treatment period of observation (tenth phase) all procedures carried out during the base-line observation period were repeated.

RESULTS

Effects on Psychiatric Parameters

Group 1. The depression of all 16 patients, regardless of the compound they received, lifted after varying periods of drug administration. Four of the cases in the subgroup of patients who were successfully treated with RO 4-1027 had not lost their depression when they received iproniazid in a previous study. Five patients, 2 in the iproniazid subgroup and 3 in the RO 4-1027 subgroup, showed sufficient improvement to be considered a social remission. Of these, only 3 patients, 2 who had received iproniazid and 1 who had been treated with RO 4-1027, expressed interest in leaving the hospital.

During treatment, all 16 patients showed reduction of both motor and psychomotor retardation, and 11 patients demonstrated a change in social withdrawal. Of the latter, 5 patients were in the iproniazid subgroup and 6 in the RO 4-1027 subgroup.

Four patients who received iproniazid experienced a recurrence of a previous psychotic pattern of behavior. These psychic aberrations were classified as manic, paranoid, and excited-nihilistic reactions, and as schizophrenic regression, respectively. In the patient with the excited-nihilistic reaction medication was discontinued on the fourteenth day of the treatment period.

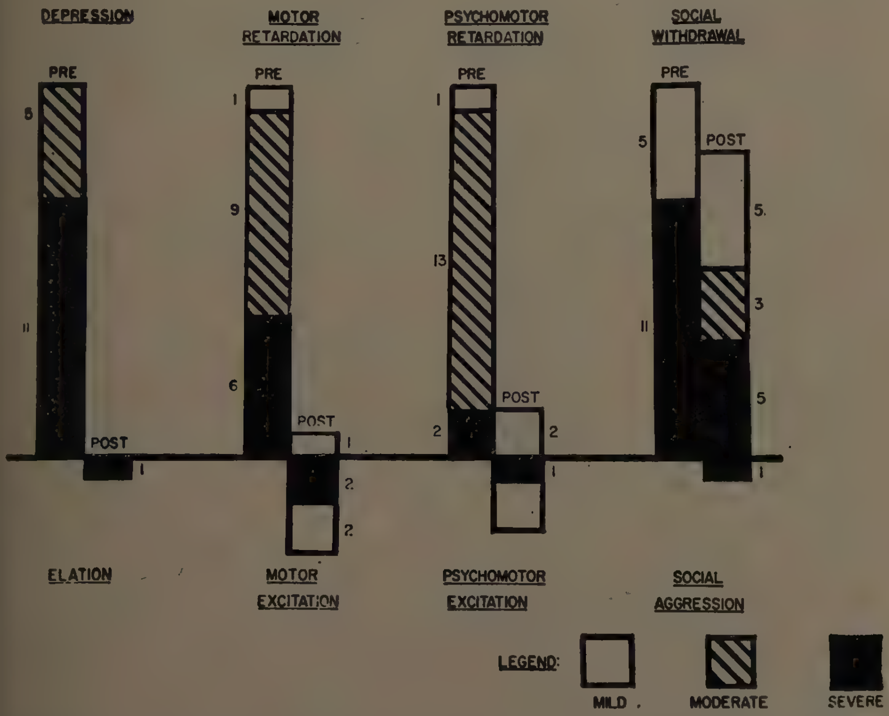

FIGURE 1. Changes of depression, motor, and psychomotor retardation and of social withdrawal of 6 patients treated with iproniazid and 10 patients treated with RO 4-1027 (Group 1).

There were some differences in terms of onset of changes between the subgroups receiving the 2 psychostimulants, but there was not a statistically significant difference between the results in these subgroups in the final evaluation.* Therefore, both subgroups were combined for the over-all appraisal of the effects of the 2 drugs. FIGURE 1 illustrates the number of patients exhibiting various degrees of affective depression, motor retardation, psychomotor retardation, and social withdrawal before initiation of treatment and after its termination. These evaluations were abstracted from the data collected through the rating procedures. As may be seen, prior to treatment the degree of affective depression was severe in 11 patients and moderate in 5. After treatment, no patient was depressed and 1 patient was elated. Prior to treatment, the degree of motor retardation was severe in 6 patients, moderate in 9, and mild in 1. After treatment, 1 patient was mildly retarded and 4 were overactive. Prior to treatment, the degree of psychomotor retardation was severe in 2 patients, moderate in 13, and mild in another. After treatment, 2 patients were mildly retarded and 3 exhibited psychomotor acceleration. Prior to treatment, the degree of social withdrawal was severe in 11 patients and moderate in 5. After treatment, 5 severely withdrawn patients remained essentially unchanged. Three patients were moderately withdrawn, 5 were mildly withdrawn, and 1 had become annoyingly aggressive.

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* The Kruskal-Wallace Analysis of Variance Technique was used to compare the separate groups on the clusters of the Wittenborn Psychiatric Rating Scale.[7]

Group 2. During treatment 19 patients showed reduction of motor retardation and 18 of psychomotor retardation. Of the former, 9 patients received iproniazid and 10 RO 4-1027; of the latter, 8 patients were in the iproniazid subgroup and 10 in the RO 4-1027 subgroup. Eleven patients demonstrated improvement in social withdrawal and, of these, 4 were treated with iproniazid and 7 with RO 4-1027. However, a social remission was not achieved in this group.

Two patients who received RO 4-1027 developed schizophrenic excitement. They both had previously experienced this psychotic pattern of behavior.

Again, there were some differences in terms of onset of changes between the subgroups receiving the 2 psychostimulants, but there was not a statistically significant difference between the results in these subgroups in the final evaluation. Therefore, as in Group 1, both subgroups were combined for the over-all appraisal of the effects of the 2 drugs tested.

FIGURE 2 illustrates the number of patients in Group 2 exhibiting various degrees of motor and psychomotor retardation and of social withdrawal before the initiation of treatment and after its termination. Prior to treatment, the degree of motor retardation was severe in 11 patients and moderate in 9. After treatment 1 patient who had been moderately retarded did not show any essential change, but 19 exhibited some degree of change. Of these, 4 were moderately retarded, 3 were mildly retarded, and 4 were overactive. Prior to treatment, the degree of psychomotor retardation was severe in 15 patients and moderate in 5. After treatment, 2 patients were severely retarded, 10 were moderately retarded, 6 were mildly retarded, and 1 exhibited psychomotor acceleration. Prior to treatment, the degree of social withdrawal was severe in 18 patients and moderate in 2. After treatment, the degree of withdrawal was severe in 9 patients, moderate in 6, and mild in 4.

Effects Other than on Psychiatric Parameters

For the purpose of evaluating effects other than on psychiatric parameters, the patients in Group 1 and Group 2 who received iproniazid and the patients in these groups who were treated with RO 4-1027 were considered together.

Of the 15 patients who were given iproniazid, 11 (73.3 per cent) gained weight, and 3 (20 per cent) lost weight. The weight gains and losses ranged from 1½ to 14 lb. and from 3½ to 11 lb., respectively.

Of the 21 patients who were treated with RO 4-1027, 14 (66.6 per cent) gained weight, and 4 (19 per cent) lost weight. The weight gains and losses ranged from 3¼ to 11½ pounds and from 1½ to 3 lb., respectively.

All 25 patients who gained weight reported an improvement of appetite and of feeling of fatigue.

The most commonly encountered side effect was a reduction of blood pressure. A drop in the mean systolic and diastolic pressures of at least 10 points, lasting for a period of 5 days or longer, occurred in all 36 cases, both in the supine and standing positions. In fact, one third of these patients, standing, developed mean systolic pressures below 100 mm. Hg and mean diastolic pressures below 60 mm. Hg. The hypotensive reaction was so severe in 3 patients treated with RO 4-1027 that it was necessary to discontinue medication on the fourteenth, seventeenth, and thirty-sixth days of the treatment period. In 2 of these patients a peripheral vascular collapse occurred; in one patient this was associated with bradycardia and hypothermia.

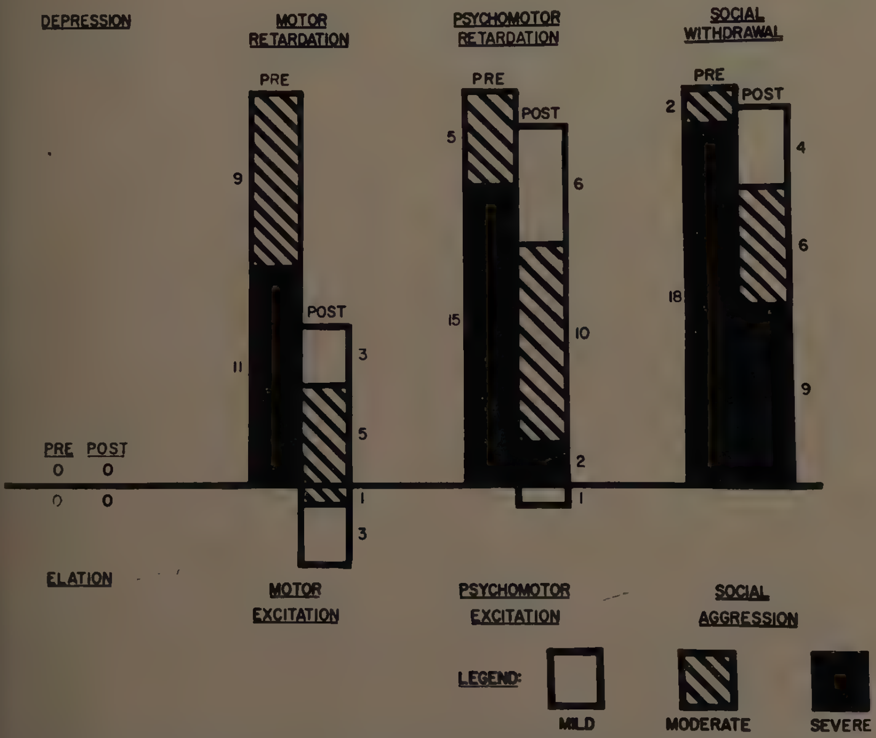

FIGURE 2. Changes of motor and psychomotor retardation and of social withdrawal of 9 patients treated with iproniazid and 11 patients treated with RO 4-1027 (Group 2).

Dizziness occurred in 5 of the patients who were treated with iproniazid (33.3 per cent) and in 11 of those who received RO 4-1027 (52.4 per cent). The dizziness appeared between the twelfth and twenty-eighth days of the treatment period and tended to persist. Although it usually coincided with the fall in blood pressure, this appeared to be an individual rather than a constant relationship.

Gastrointestinal side effects included constipation, anorexia, and nausea. Constipation occurred in 4 patients treated with iproniazid (26.6 per cent) and in 5 treated with RO 4-1027 (23.8 per cent). All of these patients had a history of constipation of many years' duration.

TABLE 2

SIDE EFFECTS OCCURRING DURING TREATMENT PERIOD OF 15 PATIENTS RECEIVING IPRONIAZID AND 21 PATIENTS RECEIVING RO 4-1027

Signs and symptoms	Total number of patients	Number of patients on Marsilid	Number of patients on RO 4-1027
Reduction of mean systolic blood pressure supine	36	15	21
Reduction of mean diastolic blood pressure supine	36	15	21
Reduction of mean systolic blood pressure standing	36	15	21
Reduction of mean diastolic blood pressure standing	36	15	21
Anemia	32	12	20
Weight gain	25	11	14
Dizziness	16	5	11
Constipation	9	4	5
Restlessness	9	4	5
Motor excitation	8	3	5
Weight loss	7	3	4
Recurrence of previous psychotic pattern	6	4	2
Insomnia	5	2	3
Headache	4	0	4
Psychomotor excitation	4	2	2
Anorexia	3	1	2
Confusion	3	2	1
Muscle pain	2	0	2
Nausea	2	0	2
Peripheral vascular collapse	2	0	2
Tearing	2	1	1
Blurred vision	1	0	1
Bradycardia	1	0	1
Edema	1	0	1
Elation	1	1	0
Hypothermia	1	0	1
Social aggression	1	1	0
Tinnitus	1	0	1
Vertigo	1	0	1

Pain without tenderness developed in the back and posterior quadriceps muscles of 2 patients treated with RO 4-1027.

Untoward hematic effects were observed as follows: a reduction in both erythrocyte count and hemoglobin concentration occurred in 12 patients treated with iproniazid (80 per cent) and in 20 patients who received RO 4-1027 (95.3 per cent). The decrease in the red cell count of the iproniazid-treated patients ranged from 0.28 to 0.8 million and that of the patients who received

RO 4-1027 from 0.6 to 1.68 million. The corresponding reduction in hemoglobin ranged from 0.1 to 2.3 gm. and from 0.5 to 3.3 gm., respectively. In one of these patients who had an anemia of 13 years' duration (initial hemoglobin concentration 3 gm.) and was treated with iproniazid, medication was discontinued on the twenty-fifth day of the treatment period when her hemoglobin had dropped to 2.2 gm. Incidentally, psychiatrically she had made a a complete remission and was 1 of those patients who expressed interest in leaving the hospital.

The erythrocyte counts and hemoglobin concentrations rose to base-line levels in all instances following termination of treatment.

The urinalyses of the patients treated with either drug did not show any changes except for the occurrence of urobilinogen in 2 patients who were treated with RO 4-1027. Twelve patients on iproniazid and the same number on RO 4-1027 showed increases in their cephalin flocculation tests of 1 plus to 4 plus, but 9 of these patients treated with iproniazid and 10 treated with RO 4-1027 had shown similar increases during the base-line observation period.

None of the 36 patients developed clinically demonstrable evidence of renal or hepatic pathology.

The psychiatric and somatic side effects described above, as well as some other minor reactions encountered during the treatment period by the 15 patients who received iproniazid and the 21 patients who were given RO 4-1027, are listed in TABLE 2 in order of their frequency.

One patient in Group 1 who had been treated with iproniazid experienced a *grand mal* convulsion on the fourth day of the post-treatment period of observation. He had had a transorbital lobotomy 2 years prior to the study period.

COMMENTS

It is obvious from the data presented that both iproniazid and RO 4-1027 produced significant and pronounced changes in both groups of patients studied. Thus, all 16 patients with a diagnosis of manic-depressive, psychotic reaction: depressed type, lost their depression regardless of which of the 2 psychostimulants they received. Furthermore, these 16 patients and 19 patients with a diagnosis of schizophrenic reaction showed a reduction of motor retardation. Similarly, a reduction of psychomotor retardation occurred in all patients with chronic depression and in 18 of the 20 patients with chronic schizophrenia. Again, both drugs were about equally responsible for producing these changes. Finally, 11 patients with chronic depression and approximately one half of the patients with schizophrenia showed improvement in their social withdrawal, and there was no gross difference in the activity of iproniazid and RO 4-1027 in producing changes in this area. Nevertheless, the impression was gained that on a weight basis RO 4-1027 is more effective than iproniazid, since 4 patients with chronic depression who had not responded to iproniazid in a previous study were successfully treated with RO 4-1027 in the present study. Moreover, it appeared that the incidence and severity of some side effects following administration of RO 4-1027 were somewhat greater than after iproniazid therapy. Thus, the hypotensive reaction that developed in all patients was so severe in 3 cases treated with RO 4-1027 that it was necessary to discontinue medication. Similarly, dizziness and anemia occurred more frequently with RO 4-1027.

Both drugs produced the same types of side reactions which, in general, did not require measures other than symptomatic management. However, medication was discontinued earlier than originally planned in a total of 5 patients, namely, the aforementioned 3 cases that developed severe hypotension on RO 4-1027 and 2 patients on iproniazid, of whom 1 developed an excited-nihilistic reaction and the other a severe anemia.

It is worthy of note that only 3 of the 5 patients with chronic depression who were considered a social remission expressed interest in leaving the hospital, but 2 did not. Our colleague N. D. C. Lewis reviewed these patients and suggested the term symbiosis as descriptive of their dependent relationship with the hospital at a time when their depression was no longer present.

In contrast to the group with chronic depression, none of the patients with schizophrenia achieved a remission. There was no significant change in the direction of appropriate affect, thought content, or insight. Undoubtedly, both the chronicity and the essential nature of the personality disorder affected the over-all response.

The results obtained in this study attest to the feasibility of screening the comparative merits of two psychostimulants in a medium-size series of patients observed over a relatively short period of time. The design used should be applicable also to comparative studies of other psychopharmacological agents, with the recognition that a longer period of medication might add to both the number and degree of changes and side effects.

SUMMARY

Iproniazid (Marsilid) and one of its analogues, RO 4-1027, which chemically is 1-(*p*-chlorobenzoyl)-2-isopropylhydrazine, were studied in 16 patients (Group 1) with a diagnosis of manic depressive, psychotic reaction: depressed type, and in 20 patients (Group 2) with a diagnosis of schizophrenic reaction. In Group 1, 6 patients were treated with iproniazid and 10 with RO 4-1027. In Group 2, 9 patients received iproniazid and 11 the analogue.

During a premedication observation period of 5 days a base line was established for each patient. This included a psychiatric evaluation, determination of eating, sleeping, and bowel habits, of interest in ward activities, of weight, body temperature, pulse rate, respiratory rate, and of supine as well as orthostatic blood pressures and, finally, several laboratory tests.

Both medications were given according to the same schedule, namely, 75 mg. daily for 5 days, 150 mg./day for 30 days and 75 mg./day for 5 days, for a total treatment period of 40 days. However, 5 patients, 2 on iproniazid and 3 on RO 4-1027, did not complete the entire course. In these instances medication was discontinued because of severe side effects.

During the treatment period the changes in degree of affective depression, of motor and psychomotor retardation, and of social withdrawal, were evaluated daily. Moreover, the Wittenborn Psychiatric Rating Scale and the Symptom Profile were completed midway in the treatment period. In addition, the

changes in all other parameters studied during the base-line observation period were determined at strategic intervals during the treatment period. The post-treatment period of observation included similar procedures.

Both iproniazid and RO 4-1027 produced pronounced changes in both groups of patients. Since there was not a statistically significant difference between the effects of the two drugs in either group of patients, the results obtained with iproniazid and with RO 4-1027 in Group 1 on the one hand and in Group 2 on the other were combined for the over-all evaluation of effects which were as follows:

All 16 patients in Group 1 lost their depression, and 5 of them achieved a social remission.

All 16 patients in Group 1 and 19 patients in Group 2 showed reduction of motor retardation.

The psychomotor retardation of all 16 patients in Group 1 and of 18 patients in Group 2 was reduced.

Eleven patients in Group 1 and the same number in Group 2 demonstrated a change in social withdrawal.

Four patients in Group 1 and two in Group 2 experienced a recurrence of a previous psychotic pattern of behavior.

A social remission was not achieved in Group 2.

The effects of the 2 drugs in nonpsychiatric areas were evaluated separately for iproniazid and for RO 4-1027.

The most commonly encountered side effect was a hypotensive reaction that occurred in all 36 patients. In 3 patients treated with RO 4-1027 this was so severe that it became necessary to discontinue medication. Dizziness occurred less frequently with iproniazid than with its analogue. The incidence of anemia was somewhat higher, and its severity was greater with RO 4-1027 than with iproniazid. However, with both drugs the red blood picture returned to base-line levels during the post-treatment period of observation. The somewhat greater side effect liability of RO 4-1027 and the fact that 4 patients with depression were successfully treated with this drug, but had not responded to iproniazid in a previous study, support the impression that, milligram for milligram, RO 4-1027 is somewhat more potent than iproniazid.

It is concluded that the method employed lends itself to the comparative screening of psychostimulants.

Acknowledgments

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References

1. Crane, G. E. 1956. The psychiatric side effects of iproniazid. Am. J. Psychiat. **112:** 494.
2. Wiedorn, W. S. & F. Ervin. 1954. Schizophrenic-like, psychotic reactions with administration of isoniazid. A.M.A. Arch. Neurol. Psychiat. **72:** 321.
3. Kline, N. S. 1958. Clinical experience with iproniazid (Marsilid). J. Clin. Exptl. Psychopathol. **19:** (Suppl. 1) : 72.

4. Loomer, H. P., J. C. Saunders, & N. S. Kline. 1957. A clinical and pharmacodynamic evaluation of iproniazid as a psychic energizer. Psychiat. Research Rept. **8:** 129.
5. Freymuth, H. W., H. Waller, P. Baumecker & H. Stein. Clinical observations of the effect of iproniazid (Marsilid) on chronic and regressed schizophrenics. Diseases of Nervous System. In press.
6. Wittenborn, J. R. 1955. Wittenborn Psychiatric Rating Scales. Psychological Corp. New York, N. Y.
7. Walker, H. M. & J. Lev. 1953. Statistical Inference. Holt. New York, N. Y.

Discussion of the Paper

Sidney Merlis (*Central Islip State Hospital, Central Islip, N. Y.*): The paper presented by Tobin and his colleagues, the first effort by the New Jersey Research Group, is an auspicious beginning. The over-all approach has been to establish adequate controls, base-line studies, and what I consider an excellent research design. It is important to realize that there are many pitfalls inherent in the evaluation of psychopharmacological compounds, and even the best-thought-out research projects cannot be completely sound and free from extraneous uncontrollable factors. We have reached the point in psychiatric research where a simple clinical impression in terms of drug effect is not always sufficient to satisfy scientific requirements. Well-thought-out research designs help to minimize, to some degree at least, the difficulties in evaluating patients on specific drug therapies. While it may seem unnecessary at first to set up base lines and establish questionnaires, it is the only satisfactory way under our present circumstances to obtain data that can be verified by something more than simple clinical impressions. Several comments are in order regarding the findings presented.

First, Tobin and his group are, I believe, quite right in stating that the incidence of true depressions in a chronic hospital population is relatively infrequent. At present, simple depressions that appear to respond adequately to the antidepressant drugs are most often treated on an outpatient basis and are not often found in the chronic institutionalized group. This may account for some of the discrepancies in the results reported by various investigators using these compounds. It is conceivable that, since the patient material under study varies, the results will therefore also vary. This is another reason for careful establishing of base-line standards and adequate research designs. Another fact to be noted is that a large percentage of patients previously maintained on other psychotropic drugs showed abnormal liver function prior to the study with iproniazid (Marsilid) and its analogues. I am uncertain as to why this should be. Reviewing our laboratory studies in patients at Central Islip State Hospital treated with various drugs, we have found a small incidence of liver-function abnormalities.

The results that Tobin has found with these drugs are in agreement with those of most other investigators. I have yet to clarify my own thinking regarding the therapeutic significance of increasing psychomotor activity induced by these drugs. Frequently this action merely serves to intensify psychotic behavior; often patients are "activated" and behave as if driven in an uncomfortable manner without demonstrating true therapeutic effect. Nevertheless, in our present state of knowledge, it appears that in such patients the antidepressant drugs or psychic energizers serve as a pivotal point from which ancillary therapies may be instituted to bring about clinical improvement. I feel certain that Tobin shares this attitude.

The toxic effects presented in this report again demonstrate that iproniazid is to be used with caution. While some of us may either ignore the significance of side effects or minimize their incidence, one must in all honesty accept the fact that these compounds have a rather narrow margin of safety. Under the present circumstances I should be reluctant to utilize them in outpatient practice where adequate laboratory and clinical control is frequently lacking. At present, I consider iproniazid and its analogues as extremely interesting drugs that exert an unusual physiological action. This action can serve as a most useful research tool in helping to elucidate some of the factors involved in emotional disturbance. Until drugs with far less intense and frequent side effects are introduced, the use of these compounds should best be limited to investigators with adequate laboratory and clinical facilities for handling the problems associated with the use of these compounds.

Theodore R. Robie (*Hospital Center, East Orange, N. J.*): The muscle pains that Tobin saw in his patients were undoubtedly due to pyridoxine deficiency and can be avoided by administering pyridoxine to all patients given iproniazid.

I am sure that many will agree that a good number of the side effects reported by Ayd are basic symptoms of depression itself; the more serious hypotensive effects could have been avoided by controlling the dosage carefully.

I cannot agree with the suggestion of Merlis that amine oxidase inhibitors should be given only to hospitalized patients any more than I should have accepted the same dictum regarding electroshock therapy (EST) 10 years ago. The fact that today as much or more EST is given in doctors' offices as in hospitals fully corroborates that position.

With the exception of that of Nathan Kline, most of the research knowledge that brought iproniazid (Marsilid) into prominence has been contributed by doctors in private practice. With new developments in the making, it seems quite likely that physicians in private practice, who have more at stake, will stimulate the laboratories to help make this form of chemotherapy safer.

Referring to the point made by Yanof, there is a good chance that Felix Wroblewski's contribution will become invaluable. There seems to be evidence that his transaminase test will tell much faster than any other known method how to protect patients against toxic effects. This test measures three things: (1) necrosis of cardiac muscle, (2) of skeletal muscle, or (3) liver damage. If the first two are ruled out then a high test is presumably due to toxic effect on the liver, although this may not necessarily be caused by iproniazid. When transaminase levels are elevated, amine oxidase inhibitors are quickly stopped. In the short time that I have been using the Wroblewski test I have attained a much greater measure of certainty regarding dosage.

RELATIONSHIP OF IPRONIAZID TO EPINEPHRINE METABOLISM

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Introduction

The antitension, antidepression, or energizer activity of iproniazid may be due to an accumulation of amine oxidase substrates. Either the catecholamines, serotonin, or both may be involved. Since the catecholamines have a long and honorable association with anxiety, tension, and "flight or fight" mechanisms, it is logical to examine the role of the amines before heavily involving the relative late-comer, serotonin.

Biochemical hypotheses of mental disease are most essential for the progress of psychiatry. It is conceivable that psychiatrists of the future will be more familiar with biochemistry than they are today. However, careful psychiatric observers have accumulated a vast body of data that must not be glossed over lightly by biochemists and neuropharmacologists. Biochemical hypotheses must win psychiatric recognition before they can be employed usefully, and this they will not do unless they take into account well-established relationships among personality, mood, and stress.

The striking effect of iproniazid (Marsilid*) on mood suggests such a relationship, but it may be mediated through any amine oxidase substrate. In order to examine this further, I have listed in TABLE 1 some categories of animal and human response that are, in my opinion, related to mood.

Neither serotonin nor norepinephrine is related to anxiety and tension, nor do they produce euphoria. On the other hand, epinephrine infusions following treatment with iproniazid produced euphoria in 8 of 10 volunteers, (Friend, 1958). Apparently, iproniazid converts the tension of the epinephrine into euphoria, since epinephrine alone aggravates anxiety.

The closest approach to human euphoria in animal experimentation is the positive-reward self-stimulation experiment. It may be safely assumed that, for these animals, substances that increase the rate of self-stimulation are euphorizing drugs; at least, they come back for more. Olds and Olds (1958) and Eiduson (1958) found that iproniazid was positively rewarding, as was epinephrine, but neither serotonin, its precursor, nor norepinephrine produced any reward. Olds and Olds concluded that iproniazid "is an excitant of reward functions in this motivational system of the hypothalamus and that quite probably it has this excitatory function in common with epinephrine."

Finally, neither serotonin nor its precursor antagonized reserpine-induced inactivity in animals, whereas dihydroxyphenylalanine (DOPA) and the catecholamines very quickly did so (Everett and Toman, 1958); thus, there is no apparent relationship between 5 psychological and activity parameters and serotonin. Norepinephrine is related to 1 and epinephrine to all 5 of them.

5-Hydroxytryptophan produces excitement in some animals, but animal hyperactivity is not the same as human euphoria. This is a fallacy frequently inferred in the pharmacological literature, where it is assumed that animal inactivity is equivalent to tranquility in humans, and animal overactivity is analogous to euphoria; the continuum euphoria-to-depression may be quite independent of the continuum overactivity-to-underactivity. Deep depression may occur with stupor or great excitation. Euphoria may be associated with complete inactivity, as in the mystical experiences induced by lysergic acid diethylamide (LSD-25) or with overactivity of the manic state. Thus, there is little evidence that serotonin metabolism is related to mood, although it would not be surprising if it played some part in brain function.

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* Hoffmann-La Roche.

It is not likely that the accumulation of epinephrine in itself is responsible for euphoria, for excess epinephrine in the brain ventricles induces anesthesia (Leimdorfer and Metzner, 1949). More probably by blocking amine oxidase, iproniazid forces epinephrine into another pathway. Axelrod *et al.* (1958) reported that iproniazid increased the conversion of epinephrine into methoxy epinephrine in rats from 25 to 50 per cent. Other possible pathways might lead to the production of epinephrine sulfate or to adrenochrome and to an indole family of compounds. The euphorient activity of iproniazid may therefore be due to a diversion of epinephrine or methoxy epinephrine to adrenochrome or its derivatives.

TABLE 1

RELATION OF SEROTONIN, NOREPINEPHRINE, AND EPINEPHRINE TO MOOD

Response	Serotonin	Norepinephrine	Epinephrine
1. Anxiety	No	No	Yes
2. Euphoria	No	No	Yes
3. Positive reward	No	No	Yes
4. Positive reward after iproniazid	No	No	Yes
5. Antagonism of reserpine depression	No	Yes	Yes

In 1957 I suggested that anxiety and tension are functions of the ratio epinephrine to adrenochrome and its family of compounds. If this is how iproniazid functions, it follows that adrenochrome and its family of compounds are antitension or euphorient compounds, and that iproniazid may increase the formation of adrenochrome or alter its destruction as does LSD-25 (Hoffer, 1958a, 1958b). We have therefore examined both these possibilities.

Antitension Properties of Adrenochrome and Adrenolutin

The antitension properties of adrenochrome and adrenolutin were observed in our early experiments with these substances. The most common reaction to these compounds was a remarkable decrease in tension. One skilled psychiatrist was given adrenolutin orally and expected an LSD-like reaction. He reported that "over the next twenty minutes or so the anxiety steadily decreased until about 6:30 P.M. when it was replaced by a feeling of complete indifference. This feeling is difficult to describe. It was unlike anything I have experienced before. At 7:00 P.M., I went to the EEG department. I remembered contrasting this visit with the one in the afternoon before I took the drug. During the afternoon, I chatted with the technician and was interested in all that was going on; now as a person he hardly existed for me. As he moved my head around to apply the band, I remember thinking that I was like one of the monkeys treated with Serpasil in a recent Ciba film. During the time the EEG was taken, I lay perfectly relaxed." Before the experiment, the subject was so tense that he had a marked tremor of his hands and could hardly lift the beaker to his mouth.

Recently we administered 10 mg. of crystalline D-adrenochrome synthesized in our laboratory to 26 patients from the psychiatric wards. No information was given to the patients except that this was a routine diagnostic test. The psychological response for the remainder of the day was observed. The results are shown in TABLE 2.

Of the 13 schizophrenic patients, there was a decrease in tension in 6, but the symptomatology was increased in 10. All 5 anxiety states were greatly eased of their anxiety, with visual changes in 2, but in another patient the relaxation was followed by an increase in depression. Two of the patients with depression became relaxed and their mood became normal. The other was more depressed. Two of the alcoholics were eased of their tension, but visual and other changes occurred in 3 others.

TABLE 2
EFFECT OF D-ADRENOCHROME ON CLINICAL STATE OF GROUPS OF PATIENTS

Group	N	Tension			Other changes	Visual changes
		Increase	No change	Decrease		
Schizophrenic	13	3	4	6	10	8
Anxiety	5	0	0	5	1	2
Depression	3	1	0	2	2	0
Alcoholic	5	1	2	2	3	3
Total	26	5	6	15	16	13

After the first adrenochrome injection, 6 of the subjects were given 150 mg. iproniazid for 2 days. Adrenochrome was then given for a second time: the clinical response to adrenochrome before and after iproniazid is shown in TABLE 3.

This combination of drugs made the schizophrenics more schizophrenic, but did not materially alter the clinical state of the neurotic patients. Iproniazid decreased the incidence of visual changes after adrenochrome from 4 of 6 to 2 of 8 cases.

Adrenochrome tolerance. In our laboratories, Payza and Mahon (1958) have developed a method for assaying adrenochrome in plasma, cerebrospinal fluid, and urine. This method measures adrenochrome added to plasma *in vitro* and injected into blood *in vitro.* Gershenovich (1956) and Osinskaya (1957) also have found adrenochrome and related epinephrine metabolites in tissues, while Sulkowitch *et al.* (1957) have reported trihydroxyindoles in urine.

Szara *et al.* (1958) have developed a method for extracting adrenochrome added *in vitro* to plasma. They find less than 20 μg. per liter of what they term nonspecific fluorescence. With the method of Payza and Mahon we find between 2 to 4 times as much specific fluorescence. The adrenochrome values are substantially increased by *d*-LSD-25, and methyl LSD, but not by brom-LSD and after the injection of adrenochrome.

This discrepancy in the results obtained by the 2 different methods is not surprising. It is possible that Szara *et al.* (1958), who extract plasma directly with isobutanol, fail to remove bound adrenochrome. Osinskaya (1957) reported that these epinephrine derivatives are bound to protein, and B. Melander (personal communication) found that ceruloplasmin binds adrenolutin. In the Payza and Mahon method the adrenochrome is converted in the plasma into a zinc adrenolutin complex. The new complex is probably more readily extracted by acetone.

TABLE 3
CLINICAL REACTION TO INTRAVENOUS D-ADRENOCHROME

Subject	Diagnosis	Before iproniazid		After ipronizaid		Interval symptoms
		Visual	Tension	Visual	Tension	
1	Schizophrenia	Yes	Increased	Yes	Increased	Worse
2	Schizophrenia	Yes	Decreased	No	No change	Worse
3	Schizophrenia	No	No change	No	Increased	Worse
4	Reactive depression	No	Decreased	No	Increased	No change
5	Hysteria	Yes	Decreased	No	No change	No change
6	Hysteria	Yes	Decreased	Yes	Increased	Better
7(a)	Reactive depression		—	No	Decreased	—
7(b)	Reactive depression		—	No	Decreased	Better

7(a) Fifty mg. iproniazid given by error 1 hour before first adrenochrome tolerance test.

Adrenochrome injected intravenously disappears rapidly until the original level is reached. The rate at which it is destroyed varies between diagnostic groups.

After taking a sample of venous blood, 10 mg. of *d*-adrenochrome is injected. Blood is then drawn into flasks containing heparin 15, 30, and 60 min. after the injection and is immediately analyzed for adrenochrome. Since we have injected authentic adrenochrome, there is no doubt that we are really measuring adrenochrome after the injection. The results of adrenochrome tolerance tests are shown in FIGURE 1.

Normal subjects destroy injected adrenochrome rapidly, usually within 30 min; however, schizophrenics are not able to destroy adrenochrome as quickly. Adrenochrome levels are much higher at 15 min. and, at the end of the hour, are still twice the original levels. Patients who suffer from tension or anxiety destroy adrenochrome more rapidly than do normal subjects. Similarly, Jantz (1956) reported that serum from alcoholics metabolizes adrenochrome to adrenolutin more quickly than normal serum.

Certain compounds known to influence mood markedly also alter the adrenochrome tolerance curve. The effects of LSD-25 on the destruction of adrenochrome are shown in TABLE 4.

Pretreatment with 35 μg. of LSD-25 converts a normal tolerance curve into one which resembles that found in schizophrenics. Pretreatment with 100 μg. of LSD-25 produces a grossly abnormal curve. Adrenochrome tolerance before and after 2 days of treatment with iproniazid is shown in TABLE 5.

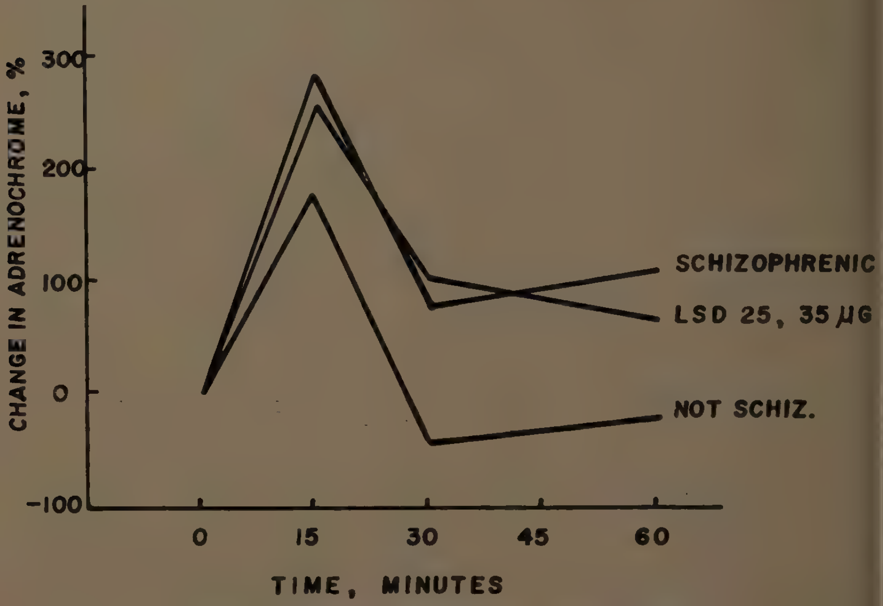

FIGURE 1. Results of adrenochrome tolerance tests.

TABLE 4

TOLERANCE FOR 10 MG. INTRAVENOUSLY INJECTED ADRENOCHROME

Group	Treatment	N	Initial level	Percentages of change at		
				15 min.	30 min.	60 min.
Not schizophrenic	None	9	51	+170	−45	−23
Not schizophrenic	35 μg. LSD-25*	6	48	+260	+100	+67
Not schizophrenic	100 μg. LSD-25*	2	46	—	—	+170

* Two hours before adrenochrome tolerance.

Schizophrenic patients before iproniazid had more plasma adrenochrome 15, 30, and 60 min. after starting the test than the group of neurotic patients. These results are in agreement with data reported by Hoffer and Payza (1958). After iproniazid, the 15-min. values were much lower.

When adrenochrome is injected into blood, the plasma blank fluorescence is increased. This is due primarily to adrenolutin. Thus, Fischer and de-Landtshoes (1950) and Fischer and Lecomte (1951) also found that adrenochrome in animals was converted into adrenolutin. The quantity of adrenolutin in plasma during this test is shown in TABLE 6.

There is very little difference in the series between schizophrenics and others. Iproniazid alters neither the conversion of adrenochrome into adrenolutin nor the rate of destruction of adrenolutin. Over the latter half of the test the adrenolutin decreased from 264 to 29, whereas after iproniazid it decreased from 250 to 73 μg./l.

TABLE 5

EFFECT OF IPRONIAZID ON ADRENOCHROME TOLERANCE

Subject	Plasma adrenochrome, μg./l.							
	Before iproniazid				After 2 days iproniazid			
	Time (min.)							
	0	15	30	60	0	15	30	60
1	96	308	—	130	123	204	115	106
2	88	240	96	181	50	93	89	33
3	91	280	80	140	100	65	125	91
4	92	250	56	77	77	180	180	100
5	67	130	96	81	94	80	58	36
6	108	200	56	64	120	187	43	61
7(a)	One hour after 50 mg. iproniazid				104	113	74	—
7(b)	—	—	—	—	78	130	87	43
Mean (excluding 7(a))	90	234	77	112	91	134	100	67

TABLE 6

EFFECT OF IPRONIAZID ON FORMATION OF ADRENOLUTIN FROM ADRENOCHROME

Subject	Plasma adrenolutin, μg./l.					
	Before iproniazid			After 2 days iproniazid		
	Time (min).					
	15	30	60	15	30	60
1	205	—	37	140	83	37
2	180	126	60	280	74	37
3	360	120	20	340	80	30
4	350	163	30	310	93	82
5	320	128	27	356	145	145
6	170	70	0	260	40	0
7(a)	—	—	—	185	110	—
7(b)	—	—	—	130	100	104
Mean	264	122	29	250	91	73

Discussion

Iproniazid increases the rate of destruction of adrenochrome in blood during the first half hour of the test, while the same quantity of adrenolutin is formed. This indicates that the adrenochrome is diverted into another pathway.

In vitro, adrenochrome in plasma is converted partially into adrenolutin. In the presence of reducing substances such as glutathione, cysteine, and ascorbic acid, some of the adrenochrome is converted into a leuko substance that undergoes further transformation. Since iproniazid markedly increases the destruction of adrenochrome without increasing the quantity of adrenolutin, it is possible that there is an increase in the formation of these leuko substances or dihydroxyindoles.

Hoffer (1957) suggested that anxiety or tension depends upon the ratio of epinephrine to adrenochrome. I now suggest that tension is related perhaps more closely to the ratio of epinephrine to some of these still unknown dihydroxyindoles. If this is the case, then iproniazid may be antidepressant or antitensive because it increases the concentration of these substances. It is likely that this occurs in the brain; since this transformation probably occurs slowly with known adrenochrome concentrations in the brain, this would account for the slow onset of action of iproniazid.

There was a curious difference in clinical response to the combination of adrenochrome and iproniazid. All the schizophrenics became clinically much worse over the 2 days they received iproniazid. Before iproniazid, adrenochrome produced relaxation, but an increase in visual changes. After iproniazid, visual changes were rare and there was little relaxation. The neurotic patients either were not changed or felt better during the 2 days of iproniazid treatment. Again after iproniazid, visual changes were less frequent. The same degree of relaxation occurred.

In subject No. 4, a case of reactive depression, the adrenochrome tolerance curve was changed from one that is within our range of normality to one resembling that seen in schizophrenia. It is possible that this occurs also in those patients who developed schizophrenic-like symptoms after receiving iproniazid for some time.

The antidepression activity of iproniazid may therefore be due to an increase in the production of dihydroxyindoles in the brain. If for some reason this does not occur, the adrenochrome metabolism may alter toward schizophrenia and induce a toxic psychosis.

Conclusion

Iproniazid increases the destruction of adrenochrome that is injected into blood. There is no change in adrenolutin production. It is therefore likely that other indoles are formed at a greater rate. The antitension of iproniazid may be related to the production of these indoles.

Acknowledgment

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References

AXELROD, J., J. INSCOE & B. WITKOP. 1958. J. Pharmacol. Exptl. Therap. **122:** 2A.
EIDUSON, S. 1958. Marsilid Symposium. Hoffmann-La Roche. San Francisco, Calif.
EVERETT, G. M. & J. E. P. TOMAN. 1958. Soc. Biol. Psychiat. San Francisco, Calif.
FISCHER, P. & L. DELANDTSHOES. 1950. Experientia. **6:** 305.
FISCHER, P. & J. LECOMTE. 1951. Bull. soc. chim. biol. **33:** 569.

Friend, D. G., M. S. Zileli, J. T. Hamlin, III & F. W. Ritter. 1958. J. Clin. Exptl. Psychopathol. & Quart. Rev. Psychiat. Neurol. **14:** Suppl. 1, 61.
Gershenovich, Z. S. 1955. Ukraine Biokhim. Zhur. **27:** 3.
Hoffer, A. 1957. J. Clin. Exptl. Psychopathol. **18:** 27.
Hoffer, A. 1958a. Brain Research Foundation. New York, N. Y.
Hoffer, A. 1958b. Am. J. Psychiat. **114:** 752.
Hoffer, A. & A. N. Payza. 1958. Am. Psychiat. Assoc. San Francisco, Calif.
Jantz, H. 1956. Zentr. ges. Neurol. u. Psychiat. **137:** 141.
Leimdorfer, A. & W. R. T. Metzner. 1949. Am. J. Physiol. **157:** 116.
Olds, J. & M. E. Olds. 1958. Science. **127:** 1175.
Osinskaya, V. O. 1957. Biochem. (Translation). **22:** 496.
Payza, A. N. & M. Mahon. 1958. To be published.
Sulkowitch, H., G. M. Perrin & M. D. Altschule. 1957. Proc. Soc. Exptl. Biol. Med. **95:** 245.
Szara, S., J. Axelrod & S. Perlin. 1958. Am. J. Psychiat. **115:** 162.

THE PHRENOPRAXIC ACTIVITY OF A NONNOXIOUS ANTIDEPRESSANT

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To describe the phrenopraxic activity of β-phenylhydrazine (phenelzine W-1544) data have been obtained through the study of patients selected according to the definitions my colleagues and I set forth in our recent publicatio “Rapid Screening of Phrenopraxic Drugs.”[1] This was necessary because the term depression lacks uniformity of meaning at present, although this need not be so, and operational definitions are needed for purposes of semantic comphrehension. In another recent paper[2] we attempted to demonstrate that “depression” need not be the hopelessly polycephalic and vague word it now is This was not our first attempt,[3, 4] nor are we alone in the demanding of a sharper semasiological profile for the different conditions involved in this syndrome one of the most recent and acute statements was that of Lehman.[5]

We can recognize acceptable parameters of the entities involved in depression This is of great importance and value in pure research, as it permits us to compare[6] the physiological and phrenopraxic spectra of different therapeutic approaches and even to evaluate the intrinsic value of a given treatment. However, we can achieve this desirable status only through the discriminating rejection of individuals whose symptomatology may not be sufficiently clear t warrant a concrete diagnosis of depression, but only an impression of it.

Because iproniazid, the original monoaminoxidase inhibitor described, had a prolonged action gradient and toxic features that rendered its use hazardous the aspects of toxicity and safety of use were prime criteria in evaluating phenelzine, the data obtained in this case allowing the practicing clinician to contro and predict its action at all times at any dosage.

In our investigations over the years we have determined that depression offer an unusually high proportion of spontaneous remissions and further, tha there are numerous instances where diagnosis cannot be clearly established consequently, our research design included provisions to exclude such spontaneous improvers, placebo reactors, and borderline cases from the stud groups.

Patients that simulate depression or who give the impression of being depressed are numerous; so numerous, in fact, that they contribute to the gros errors that militate either for or against the performance of any treatment an are reflected in the usual purportedly statistical tables we frequently see associated with reports of supposedly valuable or useful drugs. Failure to conside this group of patients in a research design leads the unwary investigator delightedly, but erroneously, to report on the activity of some compound. Thi obviously results in the plethora of valueless tranquilizers or energizers tha now abounds in the market.

The researcher must take into consideration the patients of this particula group, first, to exclude them from its research design and, second, because th practicing clinician cannot deny them treatment. Since the practicing clinicia must treat all patients who appear to be depressed, whether they are or not, or even if they do not appear depressed (depressions frequently fly false colors), we considered the question of safety to be of prime importance. This is particularly necessary in the case of monoamine oxidase inhibitors, since they have uses in many fields other than psychiatry. If our conception of their scope is correct, these inhibitors will become one of the most generally valuable drugs in the armamentarium of general practice.

In terms of dosage, a medicament is considered an absolute toxic when it engenders abnormal physiological responses with comparable dosages; a compound is considered a relative toxic when the abnormal responses are less related to dosage than to actions indirectly expressed through other mechanisms. In this latter category the most important consideration is to determine the existence of an adequate pharmacodynamic margin of safety, expressed as the capacity to obtain and maintain adequate phrenopraxic results without producing undesirable or uncontrollable toxic effects. This ability to predict and control the action of a drug at all times to insure adequate pharmacodynamic safety is essential to the practicing clinician.

Phenelzine was evaluated in 122 patients according to the procedures we have mentioned. Separate patients treated with electroconvulsive treatment (ECT) and iproniazid were used as controls, and data pertaining to the recording of depth, rate, and progression of change of objective psychiatric signs were analyzed by means of the Marcy Psychiatric Evaluation scale.[1]

As an antidepressant, our data show that phenelzine produces remissions in the depressed phase of manic-depressive psychoses and in endogenous depressions not related to schizophrenia (both larval and overt types). It is ineffective in the treatment of depressions due to stress or reactive in origin, schizophrenic pseudodepressions, neurotic dysphoric syndromes, and in organic syndromes of the brain; these are summarized in TABLES 1 and 2. These tables show that 89 per cent of the depressed affective psychosis and 82 per cent of the endogenous depressions had complete relief of their symptomatology. These remissions occurred from 3 to 14 days after the start of therapy, and maximum improvement was always noticed not later than 5 weeks after the onset of therapy. Results in individual cases are depicted in FIGURES 1, 2, and 3. This compares very favorably with electroshock. The results of electroshock, iproniazid, and phenelzine treatments are compared in FIGURES 4 and 5.

Elsewhere we have published the results of an exhaustive study of the toxicity and general physiological responses accruing to the use of this medicament and to the discussion of the nature and mechanisms of depressions.[7] To avoid repetition, we shall merely note that this compound does not effect the hemopoietic system, liver, renal, or hormonal systems. It affects the cardiovascular apparatus by inducing supine hypertension and postural hypotension at doses greater than 25 mg. orally twice daily. Hypersecretion of mucus in the gastrointestinal tract but inhibition of its formation in the nasal and bronchial passages was noticed at oral dosages of 20 mg. 4 times daily. Toxic cerebral confusion apparently due to hyperepinephrinemia were noted in all cases at levels above 50 mg. orally 4 times daily. Drowsiness and marked lethargy were noted at levels of 30 mg. orally 4 times daily (TABLE 3). Hyperexcitement from overdosage was produced only in about 10 per cent of the patients treated.

The clinical results shown in FIGURES 1, 2, and 3 which are plotted on the Marcy Evaluation Scale grids, represent the averages obtained in the treatment of different conditions.

To illustrate the course of drug treatment the following case history is presented (see FIGURE 6). R. F., a white married female suffering from recurrent episodes of a depressive affective psychosis, had required on her 3 prior hospitalizations in 1947, 1949, and 1953, 16, 18, and 16 ECT's before her depression could be relieved. Admitted to Marcy State Hospital in February 1958, she received phenelzine (20 mg. orally 4 times daily for 3 days) and developed lassitude, somnolence, and a severe postural hypotension that required bed rest for 2 weeks. In spite of this, medication was continued, and a full remission occurred by the sixth day. She was maintained on the same dosage for 4 more days as a

TABLE 1

BREAKDOWN OF THE PHENELZINE STUDY GROUPS

Categories*	Females	Males	Total	Sat. results† (per cent)	Age group (years)
Aff. psy., dep.	14	5	19	89	20–47
Overt (24)	11	15	26	78	20–84
Endogenous depression (larval)	3	9	12	86	17–53
Psychoses with C.A.S.	16	12	28	0	65–80
Schizophrenia O.P.	4	2	6	0	32–42
C.P.	10	2	12	0	36–47
C.C.	3	11	14	0	32–49
Reactive (stress) depressions	7	2	9	0	30–48

* In order to include some categories in the format of the table properly, the following abbreviations have been used: affective psychosis, depressed type = aff. psy., dep.; C.A.S. = cerebral arteriosclerosis; the schizophrenic groups are O.P. = overactive paranoids, C.P. = chronic paranoids, and C.C. = chronic catatonics.

† Satisfactory results. The percentages represent patients considered as "satisfactory results" because they returned to Grade 1 (symptom-free) on the Marcy Rating Scale.[1]

TABLE 2

ECT-TREATED GROUPS AND IPRONIAZID GROUPS

Control

Categories*	Total treated		Sat. results (per cent)	Sat. results (per cent)
Aff. psy., dep.	4F	4M	87 ECT	42 INZ
End. dep., overt	4F	7M	72 ECT	45 INZ
End. dep., larval	2F	1M	None treated	100 INZ
Psychoses C.A.S.	6F	8M	24 ECT	20 INZ
Chronic schiz.	8F	16M	25 ECT	12.5 INZ (inj.)
Reactive dep.	3F	None	0 ECT	33 INZ

The same age groups were employed as in TABLE 1, and the same diagnostic criteria are used. These patients were treated in other hospital services at the time of our own investigations, but we evaluated their progress by the same scale.

* Abbreviations: aff. psy., affective psychosis; dep., depressed; end., endogenous; C.A.S., cerebral arteriosclerosis; schiz., schizophrenia.

† See TABLE 1.

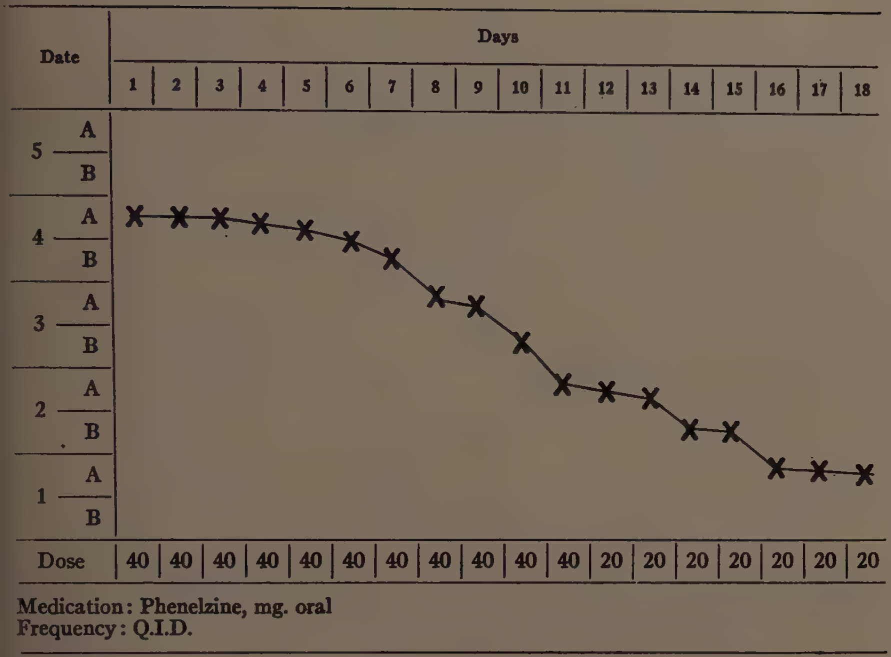

FIGURE 1. Average curve of results in treating depressed affective psychoses with phenelzine.

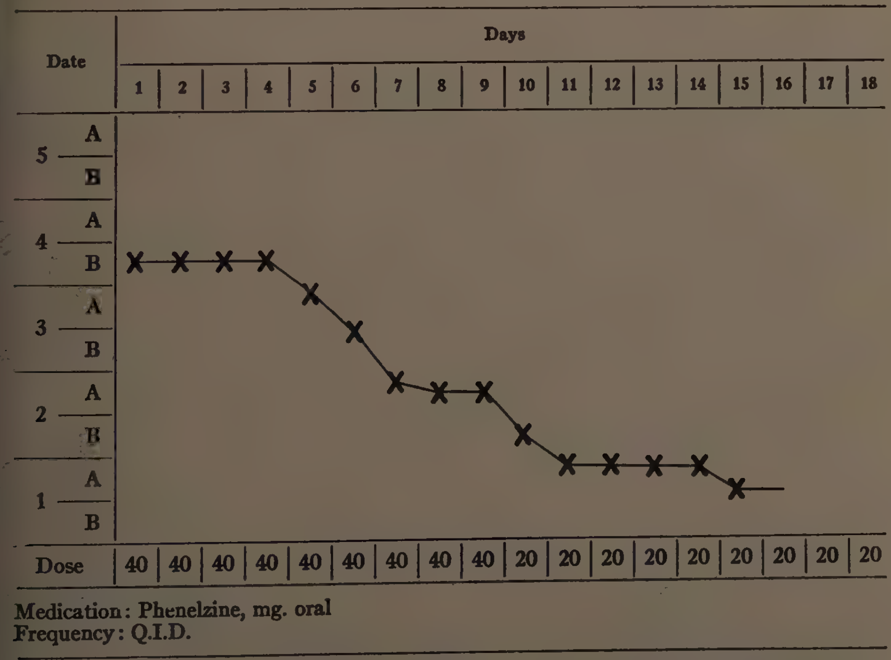

FIGURE 2. Average curve of results in treating overt endogenous depressions with phenelzine.

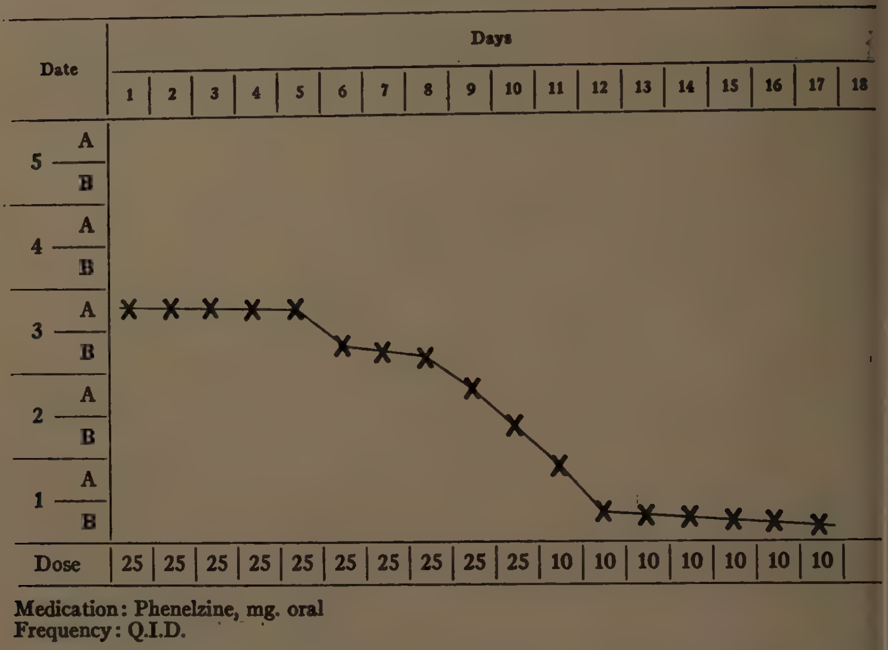

FIGURE 3. Average curve of results in treating larval endogenous depressions with phenelzine.

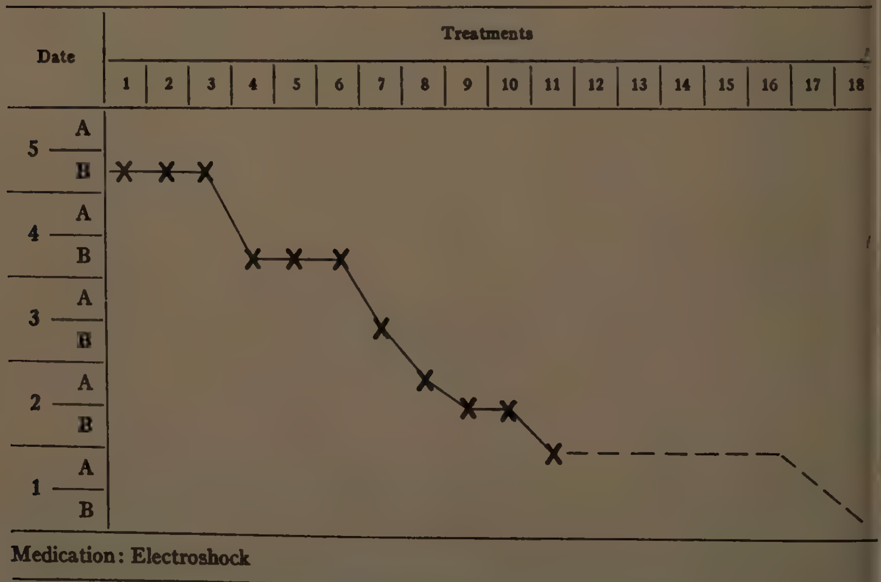

FIGURE 4. Average curve of results in treating depressed affective psychoses with electroshock, expressed in treatment days.

precaution, and then the drug was discontinued. The patient was discharged without medication because of her hypotensive tendencies. In late March 1958 she was administered parenteral phenelzine to evaluate electroencephalograph (EEG) and blood pressure responses. One interesting finding was that doses greater than the minimum hypotensive dose (MHD) of W-1544 did not produce any further increases in hypotension.

In August 1958 this patient relapsed and, although her condition was milder

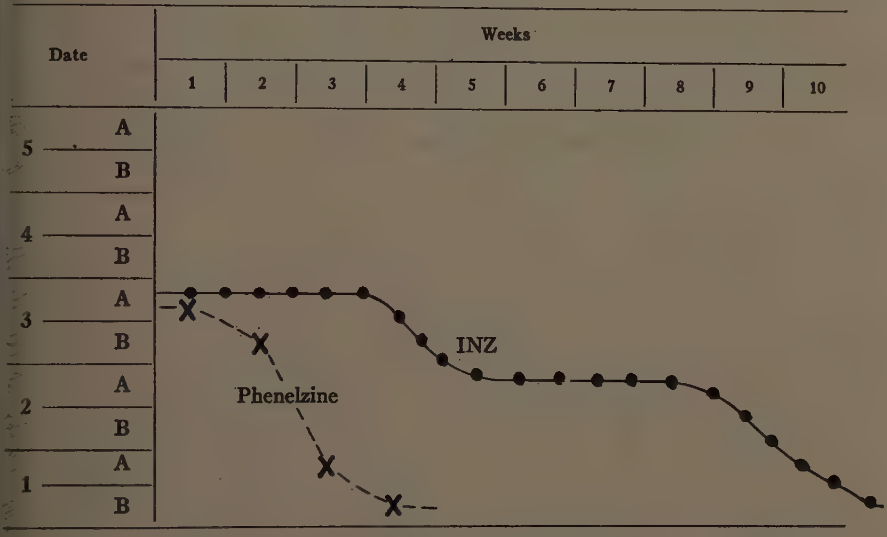

Figure 5. Comparison of the action of phenelzine (lower left curve) to that of iproniazid (upper right curve) in larval endogenous depressions.

Table 3

Summary of Side Effects Observed with Phenelzine

Side effect	20 Patients @ 40–80 mg. O.D.	72 Patients @ 80–120 mg. O.D.	18 Patients @ 120–200 mg.	12 Patients @ 200–300 mg.
Ankle edema	2	2	1	5
Hypernatremia	—	—	2	4
Drowsiness	3	30	8	6
Dizziness	—	9	—	—
Postural hypotension	—	6	2	3
Ataxia	—	—	—	2
Euphoria	—	2	1	—
Overactivity	—	—	1	—
Excitement	—	—	1	—
Hypermucosity	1	3	1	—
Nausea	1	5	1	1
Vomiting	—	—	1	2
Constipation	—	1	1	1

The side-effects encountered during our study of phenelzine (W-1544), are grouped here for convenience under the dosage groups where they were found. The percentage incidence is not expressed, having been the subject of another paper.

in intensity than that occasioning her admission in February, it was such that the need for hospitalization was postulated. To investigate whether the drug could produce another remission and whether home treatment was possible, a dosage of 30 mg. orally 4 times daily was given at home, and the patient and her husband were instructed on the need for her to stay in bed should hypotension occur. This side effect reappeared by the third day, but by this time depressive manifestations were reduced to the point where the patient was fully able to cooperate in her treatment; rehospitalization therefore was not necessary. The patient was reduced to 10 mg. orally 4 times daily after 10 days of initial treatment and was discontinued 10 days later. She continues to be in excellent condition and was last seen on November 6, 1958.

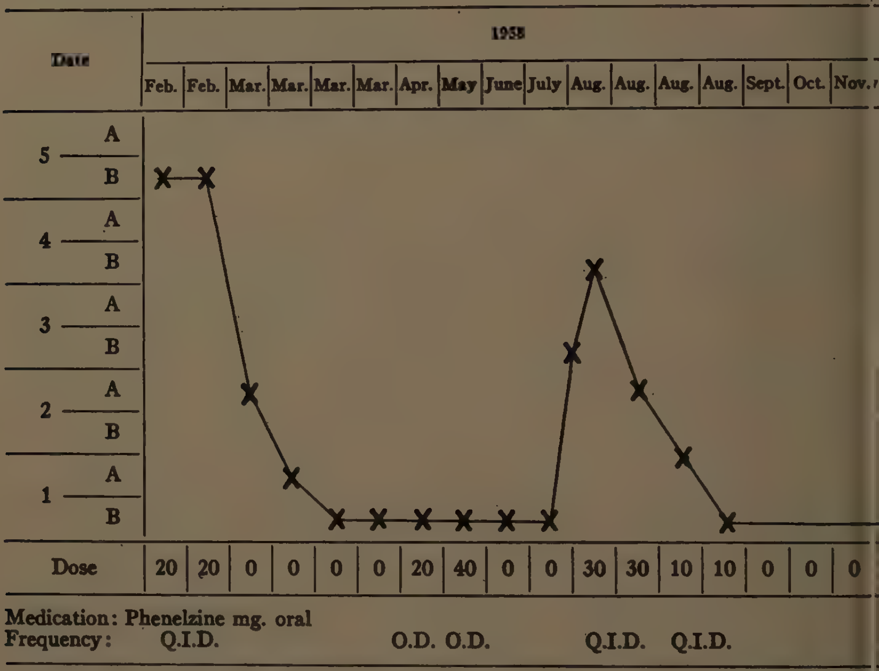

FIGURE 6. Expanded chart showing the treatment of an average manic-depressive patient.

Discussion

Our data revealed lack of correlation between inhibition of jugular vein monoamine oxidase inhibition, increases in catecholamine levels, and therapeutic results in the sense that doses of phenelzine could produce remissions without disturbing the blood levels of the substances. This does not rule out intracerebral changes not perceived by this method but, taken in conjunction with our previous findings[2, 3, 7] that depression may be reversed by drugs unable to affect monoamine oxidase, leads to doubt that the mechanism of monoamine oxidase inihibition and its resultant increases in catecholamines and serotonin levels is the only or even the primary mechanisms in the prevention or reversal of depression.

The fact that W-1544 is very selective in the inhibition of this enzyme has allowed the study and further corroboration of our theory, but does not exclude the possibility that monoamine oxidase inhibition may play a substantial role. Phenelzine also appears to be the drug that most clearly compares to electroconvulsive therapy in scope, rapidity of action, and freedom from serious side-effects.

The material described above clearly demonstrates that W-1544 is effective against the described depressive conditions. Separate studies not yet completed indicate preliminarily a dramatic response to it by patients suffering from rheumatoid arthritis. This returns us to the problem of safety in administration, because this drug and similar ones will be widely employed in general practice.

Our data led us to assume that W-1544 is less toxic than iproniazid because no hepatic, hematopoietic or central nervous system parenchymatous damage has occurred or even been foreshadowed. However, W-1544 is an absolute toxic in dosages greater than 50 to 75 mg. orally 4 times daily, producing the entire syndrome of hyperepinephrinemia. In this relation we have found that mepazine (Pacatal*) 50 to 100 mg. intramuscularly 4 times daily or trifluopromazine (Vesprin†), 40 to 60 mg. intramuscularly 4 times daily may be given as an antidote orally during the course of treatment; Pacatal is preferable to other phenothiazines in this condition as it does not produce drowsiness or acathysia or enhance the depression; otherwise W-1544 is relatively toxic only. In this connection it is curious and important to report that the injectable forms of W-1544 are somewhat less likely to produce toxic reactions to a given dose than when given orally. A very clear reaction of a similar type was noticed earlier in connection with iproniazid. The latter drug produced a cerebral toxic confusional and excited state at oral dosages of 50 to 100 mg. 4 times daily in about 2 weeks' time in 4 patients. Subsequently, these patients were given the same amount parenterally for 5 weeks without producing this toxic result, which did not appear until a dose of 75 mg. intramuscularly was reached at the end of 6½ weeks; the significance of this finding is discussed below.

Iproniazid has been justly regarded as a risky and dangerous drug because it produces yellow atrophy of the liver, central hyperthermia, blood dyscrasias, and CNS parenchymatous lesions, among others. It has been an unquestionable antidepressant activity, although not as great in scope nor as rapid in effect as phenelzine or ECT. However, its detractors have missed the 2 most important points, the first only recently commented upon by Crane:[8] that its principal danger lies in not affording the user any pharmacodynamic margin of safety or, as Crane puts it, a "satisfactory psychophysiological homeostasis." The user is therefore not even given the chance with iproniazid of taking a calculated risk because its tardy, uncertain, and protracted modes of action tend to swing the patient from an atherapeutic range, through a therapeutic zone and, beyond, into an area of severe and possibly fatal complications, all stages that may be experienced while the physician is still trying to determine the optimum therapeutic dose. The second point missed, and which we have determined and heard of but have not seen described in print, is that iproniazid inhibits enzymes other than monoamine oxidase, and it is possible that its hepatotoxi activity is due to inhibition of heptatic conjugases. This opinion is somewhat reinforced by the lessening of toxic side effects when given parenterally, as it appears that more of it would reach the brain before passing through the liver in this fashion, while the opposite would result from oral administration. This effect is only barely visible in the case of phenelzine, a strict monoamine oxidase inhibitor.

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* Warner-Lambert.
† Squibb.

Iproniazid, although limited in scope, will reverse certain conditions tha may be assumed to be depressive even if they do not fit the strict categories w have employed in our studies. This again raises the question whether mechanisms other than monoamine oxidase activation are responsible for depressive syndromes other than those specified, and makes it necessary that a further unbiased study of iproniazid be made to verify or disprove these observations

Phenelzine may be assumed to be safe because it provides the necessary pharmacodynamic margin (or what Crane calls psychophysiological homeostasis) Its therapeutic range lies between 20 to 40 mg. orally 4 times daily. The recommended starting dose is 25 mg. orally 3 times daily with meals and 25 to 50 mg orally at bedtime, the larger dose being used in depressive insomnia. Dosage should not be reduced if postural hypotension appears, bed rest and, possibly hydroxyamphetamine (Paredrine*) hydrobromide being sufficient to contro the side effect until psychiatric improvement makes possible the reduction to maintainance dose, usually 10 mg. orally 4 times daily, at which time the hypotension disappears. Ankle edema may require lowering the dose or adding mercurial (but not a saluretic) diuretic.

Conclusions

Phenelzine (W-1544) has been studied in 122 patients with varied psychiatri symptomatology. An exhaustive investigation of its toxicity was conducte while rigidly controlled diagnostic categories, placebo exclusion, triple-blin placebo techniques, and controls employing iproniazid and ECT demonstrate the antidepressant and antirheumatoid action of the preparation. Primary importance was given to its safety of administration.

W-1544 has an antidepressant effect comparable in scope, speed, and safety to that of ECT.

The action of the drug has been compared to that produced by iproniazi and ECT. Its absolute toxic range lies much higher in dosage than it therapeutic range. A case was described to illustrate treatment and therapeutic dosages recommended.

Phenelzine acts faster through parenteral than through oral administration but the therapeutic response is equal.

While this drug bids fair to reduce or even supplant the use of electroshock it does not affect all depressive mechanisms. Further studies beyond th scope of monoamine oxidase inhibition are required.

* Smith Kline & French.

References

1. Sainz, A., N. Bigelow & C. M. Barwise. 1958. Rapid screening of phrenopraxic drugs. Psychiat. Quart **32.**
2. Sainz, A. 1958. Clarification of the action of successful treatments in depressions. Eastern Psychiat. Research Assoc. Meeting. To be published.
3. Sainz, A. 1958. The psychagogic action of methonalide. Round Table on Psychic-Energizers. Am. Psychiat. Assoc. Yearly Meeting. San Francisco, Calif.
4. Sainz, A. The antidepressant effects of certain phenothiazine combinations. Psychiat. Quart. To be published.
5. Lehman, H. E. 1958. Effect of a new agent in the treatment of different depressive syndromes. Round table meeting on psychic energizers. Am. Psychiat. Assoc. Yearly Meeting. San Francisco, Calif.
6. Tardy, A. & A. Sainz. Pharmacologic and physiologic study of W-1544 (Phenelzine, rapid-acting monoaminoxydase inhibitor. To be published.
7. Sainz, A., N. Bigelow & C. M. Barwise. 1957. Effective changes produced by some phenothiazine and di-phenyl derivatives in certain psychiatric syndromes. Am. Psychiat. Assoc., Psychiat. Research Repts., **No. 8.**
8. Crane, G. E. 1958. Clinical and pharmacological studies of Marsilid and theoretical considerations concerning its antidepressant action. J. Nervous and Mental Disease. **127**(3): 238–246.

Discussion of the Paper

G. J. Sarwer-Foner (*Queen Mary Veterans Hospital, Montreal, P.Q., Canada*): Sainz's paper opens by expressing the need to define depression so that it is not a "hopelessly polycephalic and vague word." He writes of the need to select individuals whose symptomatology is sufficiently clear to warrant a concrete diagnosis of depression. I quite agree, and I also consider the term psychic energizer unfortunate. The problem of using drugs in depressions is a difficult one, and some general comments about depressive conditions and the use of drugs are in order, especially in a monograph accenting monoamine oxidase inhibition.

It is imperative to define, in modalities that are meaningful to all who read them, and in terms that permit valid observations verifiable by others, what one observes in the individual patient who is suffering from the condition termed depressed. The diagnostic terms depressive reactions, psychotic depression, depression, and depressed patient, are of only relative utility in this sense.

In terms of understanding the mechanics of a depressed state, the wholly archaic and scientifically dubious terms endogenous or exogenous depressions are worse than useless. How endogenous is "endogenous?" Does the use of the word in this sense truly represent a scientific concept? Is there any scientific validity in the assumption that there is an underlying metabolic disorder etiologically responsible and often considered of a constitutionally determined nature? Or, as the corollary to this, is there a constitutionally determined personality type that is cyclothymic in the above-mentioned constitutionally determined metabolic sense and that periodically affects the patient? Also, does the use of this term rather reflect the possibility that the person who uses it in this sense has not been able to get to know the patient better and to see what events in the patient's present life or what symbolizations of the patient's past in the patient's present life are upsetting him? Is the difference between neurotic and psychotic defenses only one of degree? What about the manic-depressive psychosis?

These questions are asked with reference to the problem of detecting what happens to the patient prior to the application of a treatment procedure or the action of powerful pharmacological agents whose physiological activity must then be integrated into and measured against the pre-existing clinical picture. One thing is certain; although the monoamine oxidase inhibitors are certainly interesting and possibly represent an important step forward in the study of cerebral metabolism and, possibly, also towards an elementary knowledge of the biochemistry of emotion, it is naïve in the extreme to attribute to this one biochemical action a one-to-one direct, important, or specific action in depressions. Clinicians experienced in following the moods, behavior and, above all, the thoughts and associations, the life histories, and the ego defenses of depressed patients find it difficult to believe it to be so simple. There is too much that cannot be accounted for by this most simple and clinically naïve hypothesis.

In assessing the changes in the patient we define the patient's condition along the following lines: What are the patient's behavior and feelings, psychological state, behavioral expression of the psychological state? How does the patient use his energies? How do the various organ systems, including the gastrointestinal tract, appetite, sleep, energy level, blood pressure, pulse, respiration, and sweating, function in this state? What was the environment in which the depression occurred and the social context in which the symptoms are expressed? What symbolized material of the patient's total life history and experiences are being expressed in the current state, as measured by the above?

If the patient's psychodynamics and life history are studied along these lines, the five following factors may be seen:

(1) In all depressions there is always the loss or threatened loss of an object relationship important to the psychic integrity of the patient. This may be a real one occurring in the patient's present life or the symbolization in the current life situation of an earlier loss that had in fact occurred. In the symbolization of this loss the patient can be said to fear poverty, death, or failure, all of these having very much the same meaning of abandonment and impoverishment. The patient always feels that without the support of receiving the gratifications found in the lost object relationship, personal "intactness" or integrity as an adequate person is either gone or greatly threatened. He further feels that this loss is his own fault and of his own doing. It results because he is so "bad" or "inadequate" for his life roles that he deserves this loss.

(2) The pain experienced by the patient in response to rejection or abandonment of the lost or loved one produces intense frustration, rage, and anger. When the object perpetuating this trauma is at the same time a significant adult in the patient's past (actually, or at a symbolized level), and one whom the patient has introjected in the identificational process, it is difficult for the patient to express this anger towards this object of love and hate. This occurs because he fears that the open expression of such affects would prove his "badness" and would cause further withdrawal of love and affection and lead to further abandonment and rejection, which the patient has already experienced to some degree.

(3) The "hated" and "loved" object, or its symbolization, was introjected by the patient; therefore anger against it is turned against the patient himself, for the hated object is also living within the patient.

(4) The struggle to control himself, to swallow up, to keep the anger introjected, raises feelings and phantasies dealing with the learning of self-mastery and control. The models of introjection, with its phantasies of intake, chewing, destruction, change, incorporation, assimilation (identification), and elimination of destroyed or waste material are part of this. The need to keep in the anger and hurt, rather than let them out, and the feeling of great guilt and of "being bad" at the destructive aspects of the above all form an essential part of the clinical picture.

(5) Although the above-mentioned elements are always present, one aspect or another may be to the foreground as the principal aspect of the presenting symptomatology. However, one can usually see the manifestations of the other aspects appear as the patient either regresses or progresses out of the depression; thus the dominance of "oral" or "anal" components in the foreground of the clinical picture will vary greatly.

Some drugs of a sedative nature can be helpful in agitation or anxiety manifestations. With passive-dependent patients desiring kindness, acceptance and oral gratification, almost any drug will do. With patients at the stage of energy lack, acceptance and a stimulating drug may well do better. None of the drugs available, however, are "antidepressants." By this I mean that none seem specific for depressed states as defined above. One must also keep in mind the fact that depressions are often self limiting, and that one often sees spontaneous recovery begin in 4 or 5 weeks. It is therefore very difficult to evaluate a drug treatment that produces results in longer than 3 to 4 weeks.

In the type of patient seen with great frequency outside of the mental hospital setting (that is, in private practice, outpatient departments, or general hospital units) with largely neurotic depressions, the passive-dependent oral components are marked, frequent, and always form a prominent part of the clinical picture.

In view of the above-outlined conceptual models, to speak of monoamine oxidase inhibition as being the primary factor in depression is to take an extremely naïve and simplicistic view. Monoamine oxidase inhibition in itself can neither explain nor account for the factors. It is for the biochemist and the neurophysiologist to decide what role monoamine oxidase inhibition plays in cerebral metabolism. Such knowledge is an interesting and necessary step in neurobiochemistry. It is the role of the clinician to demand that theories, biochemical or otherwise, fit the observed and verifiable clinical facts before they can be seriously entertained.

Sainz mentioned his favorable experience with the depressed phase of manic psychoses and with endogenous depressions not related to schizophrenia, using W-1544, or phenylethylhydrazine. He mentions it as being ineffective in the treatment of depression due to stress or reactive in origin, schizophrenic pseudodepressions, certain dysphoric syndromes and organic syndromes of the brain.

We have also worked with phenylethylhydrazine in open psychiatric settings, and this work is continuing. For several months prior to the meeting, 15 patients with depressive reactions in open psychiatric settings, and 5 tubercular patients with apathy and retardation were treated in a tuberculosis sanatorium. At this writing the average duration of treatment had been 3 weeks and the average dose 40 mg./day. It was felt that this dose level was probably inadequate to obtain a clear-cut physiological action, since there was little in the way of therapeutic response. Three patients had an increase in irritability, became hyperactive, and were taken off the drug. The only side effect had been some dizziness, possibly due to postural hypertension. There was no evidence of any liver, blood, or urinary toxicity on weekly studies during this period; accordingly we too tend to think of this drug as relatively nontoxic, at least to date. At present we are in the process of increasing the dose range to 100 mg. or more daily. In the few patients with whom this has been done, greater physiological stimulation was produced. The intramuscular injection and the use of morning loading doses on a few patients very recently have produced remarkable physiological stimulation in a short period of time. This method of administration therefore is receiving serious study in our hands.

I shall conclude by congratulating Sainz on his paper, which is clear, and on the triple-blind method that he and his associates at the Marcy State Hospital have devised for testing the drug within a state hospital. This technique, which deserves to be better known, helps determine the breadth and scope of the drug effect and its use.

David C. English (*Mercywood Neuropsychiatric Hospital, Ann Arbor, Mich.*): A good discussant of a paper should not agree entirely with its author nor should he disagree entirely with him. The source of my patients, comprising an entirely private practice with no university research connection, provides an excellent contrast to the patients treated by Sainz. A further partial contrast comes from my use of iproniazid with 220 cases of agitated depression, all personally followed with good results. Most of the agitated depressions received chlorpromazine (Pacatal) for control of their initial agitation, and prevention of possible overstimulation from iproniazid or the later phenylethylhydrazine (W-1544A). For the last ten weeks, W-1544A has been used alternately with iproniazid with each being given to 40 patients. The side effects, only minor in my series of 243 iproniazid patients, seem to be probably less with W-1544A, but a series of 40 patients obviously is too small to have statistical value. In contrast to the report of Sarwer-Foner, from the use of these drugs in 286 patients, the fast and remarkable recovery of most of them, often without electroshock therapy (EST), I can only conclude that these drugs are much more than stimulants and, in fact, can almost replace EST even in a private hospital setting where time is much more important.

I also cannot agree with Sarwer-Foner's opinion that patients spontaneously recover from a depression, so that using a series of patients to compare efficacies of drugs means nothing; studies of large series of patients in private hospitals for the past 130 years have shown a relatively consistent in-hospital stay of 7 months. With the use of EST this period has been shortened to about 6 weeks, which is certainly a very great change. Using the drugs discussed in this monograph the shortening is close to that with EST.

"Depressive indices" or any standards for rating depressions produce results that appear quantitative but are still based on subjective estimates. With Sainz, I should like to believe that psychiatric research could be done on an entirely objective basis; however, no matter how many indices of patient improvement one establishes, and no matter how elaborately or statistically a given series is analyzed, the actual evaluation of an individual patient's improvement in terms of these artificial indices becomes entirely personal and subjective. I am sure that every study, my own included, contains some misdiagnosed patients: catatonic schizophrenics with only a fair prognosis mixed in with nonagitated depressions with a good prognosis is the commonest example. Such misdiagnoses probably cause minor errors in reported drug results; the majority come from an individual's own prejudices, which we all have and cannot completely escape. Although double- and triple-blind studies would seem to avoid such errors, extremely accurate and even more detailed comparisons of such studies with each other and with the usual methods all on the same drug have shown they are little more effective, if at all. Like everyone else, I have wondered how some men could get such tremendous results with a drug that did little in my hands. Even when I begin to use such a drug on a series of patients, fully believing the drug will be effective and a great therapeutic addition (that is, with a positive psychological "set") it becomes only too obvious the drug has not helped patients, and then often it gradually disappears from the market. If such a drug remains available, a fair number of physicians must be using it repeatedly, and for them at least, it must work. Very few, if any, such drugs are "useless"; however, they have uses that are much more limited than the initial extravagant claims made for them.

Iproniazid and its analogues are understood by everyone to constitute a special group of compounds that, in psychiatry at least, require a new name. "Psychic energizers," Nathan Kline's term,[1] seems to be an excellent one, especially since it obviously fits in with Freud's initial breakdown of psychoanalysis into psychoenergetics, psychotopography, and psychodynamics. As Kline has mentioned, the first part of this breakdown is very much the most neglected part of the trilogy, best witnessed by the fact that hardly anyone has heard of it; psychotopography is the weakest part, but psychodyanmics, in contrast, is so well known that many people feel that it includes, at least implicitly, the other two. Since a term is only what we define it to be, actually terms like psychic activators, encephalopraxics, or freshly invented ones such as hyperhedonigens would be as valuable, since the important thing is both for everyone to agree on a common meaning for a term and then to use it with such frequency that its meaning becomes automatic to everyone. Every clinician would like to be able to predict and control the therapeutic side effects of any drug at any time, but in practice there is a calculated risk with any and every drug.

Sainz discusses 122 patients who received varying doses of phenylethylhydrazine (W-1544A) at varying dosages, in which 89 per cent of the manic-depressive depressions and 82 per cent of the depressive psychoses showed improvements. His FIGURE 6, given at the end of the paper for the above 2 groups, however, apparently shows the endogenous group improving faster and better than the manic-depressive group, which accords with my experience in 223 depressed patients, 180 of whom were treated with iproniazid (Marsilid) and 43 with W-1544A. Sainz reported that W-1544A was ineffective in 9 cases of reactive depressions and in 14 cases of schizophrenic pseudodepressions; I believe that good results can be obtained in both groups with lower doses of iproniazid or W-1544A balanced with mepazine (Pacatal), as is explained more fully in my discussion of the presentation of Scanlon elsewhere in this monograph. However, in 60 per cent of 43 catatonic schizophrenics (7 of whom received 1544A and 36 iproniazid, all with Pacatal) I found good results. Kline has shown that these drugs "open up" schizophrenics in all stages of their illness,[1] but occasionally the increased activity far exceeds acceptable bounds; what probably is more important in my series is that the 40 per cent who did not show significant improvement did not become worse, either more abusive or more combative, because of the added tranquilizer.

Sainz reports improvement with W-1544A in 3 to 14 days, and maximum improvement within 35 days; my 43-patient series shows a maximum hospital benefit (MHB) of 26 days, which is 9 days less than in Sainz' series. The 26 days can be compared with a 27-day MHB with 69 patients receiving 150 mg./day initially of iproniazid (the bulk of Sainz' patients is broken into groups of less than 40; these and my groups on W-1544A are too small to be statistically significant). Reduction of the initial iproniazid dosage in my next 174 patients to 75 mg./day extended the median time to 30.4 days. Sainz does not mention the number of iproniazid patients or their dosage, but states that the drug produced some improvement with mild endogenous depressions in 28 days and maximum improvement by 77 days. I am sure that rating methods differ sufficiently among investigators to correlate satisfactorily my W-1544A median improvement time 26 days with his 35 days; however, I cannot explain why the Marcy Hospital iproniazid patients improved in 77 days, while the Mercywood Hospital iproniazid patients had recovered by 30 days. It certainly is true that most monoamine oxidase (MAO) inhibitors are nearly as rapid in effectiveness as electroshock therapy (EST). Although many large series (my own included) have shown that 5 to 10 per cent of cases do better with drugs than with EST (for some unknown reason), yet at least 10 per cent do much better with EST than with antidepressants and at least another 10 per cent require both for improvement.

As to side effects of W-1544A: from my series of 40 patients, the only one has been a minimal hypotension. My patients all received 100 mg. per day and showed nothing approaching the drowsiness that Sainz found in 30 of 72 patients at the same dosage level nor the nausea found in 5. Nathan Kline, in his discussion of Agin's presentation, mentioned the optimum W-1544A dosage was 60 mg./day. At this dosage level Sainz studied only 20 patients, but found ankle edema in 2, drowsiness in 3, and nausea in 1. My series of 40 patients has shown no ankle edema; however, my first case of definite pitting edema with Marsilid occurred in patient No. 189 and cleared with dosage reduction and chlorothiazide (Diuril). This patient, incidentally, remains in remission best with 25 mg. Marsilid and 100 mg. of W-1544 daily together. Either drug alone, or combinations in other dosages, either produced edema or a relapse. It is pertinent to note that even studies of side effects in groups of 200 patients are not significant: 6 cases of jaundice occurred in my first 288 patients on chlorpromazine (Thorazine). The next 300 showed none, and so it actually took a series of 600 patients to confirm the nationally accepted incidence of Thorazine jaundice.

Sainz' sample W-1544A case history developed a severe postural hypotension. As mentioned above, I have not seen this with 1544A but, if I had, I do not believe that I could sit by without reducing the dosage and attempting to elevate the blood pressure, since agitated patients are understandably very much in a panic until their blood pressure improves. The patient later relapsed, was started on a dosage 40 mg./day higher than before, and again developed postural hypotension, but was allowed to remain at home without dosage reduction. It was most informative to hear that her complications worked out satisfactorily since, in a private practice, both dosage reduction and hospitalization had seemed and still do seem imperative in such a case.

At this time, MAO inhibition seems to be the common characteristic of drugs effective in "dysphoric" ("endogenous") depressions; (2 possible exceptions are (Tofranil), which is more effective in reactive depressions, and Deaner, which is definitely effective only for reactive depressions). Kline also mentioned that the major side effects were common to all MAO inhibitors; this certainly seems likely, since it is improbable that the addition of one radical to a compound in a series would bring out entirely new reactions and totally avoid all old ones. It has seemed to me that phenethazine has qualitative side effects similar to those of the other drugs; since Marsilid also inhibits diamine oxidase and diphosphopyridine nucleotidase, it has seemed natural to assume that W-1544A has at least some effect on these enzymes also. Monoamine oxidase occurs nearly everywhere in the body; therefore side effects due to these drugs anywhere can at least tentatively be related to the MAO inhibition. In other words, a new or different enzyme need not be introduced to explain such changes as the rare liver damage that occurs with iproniazid. The only entirely unique pharmacological action of W-1544, mentioned elsewhere in this monograph by Chassin, is the nonattenuable pressor response that occurs in dogs following intravenous administration. Perhaps Sainz could mention data showing the specific and only pharmacological action that he believes W-1544A has, namely, MAO inhibition. In contrast, it appears to me that all 3 drugs (iproniazid, W-1544A, and JB-516 or β-phenylisopropylhydrazine) show approximate correlations with EST in speed, effectiveness, and side effects. The toxic reactions of all 3, as with many other drugs, are less with parenteral administration. For all 3 drugs, it may be true that the decreased parenterally induced side effects may be due to a lowered action on hepatic conjugases. There is no clinical proof, or even reason to suspect, that Marsilid has a singular action on hepatic conjugases that is not shared by other MAO inhibitors or phenacetylcarbamide (Phenurone). Whether these reactions are due to hyperepinephrinemia (as Sainz feels they are in the case of W-1544A) or following a great increase of norepinephrine in brain with all 3 drugs, they are probably due to mildly toxic effects on the same systems.

I hope that Sainz can illustrate from his cases the incidence of blood dyscrasias with iproniazid; a recent review of the entire iproniazid literature did not mention any such cases. The so-called "brain damage" and deaths reported with iproniazid probably stem from a terminal tuberculous death occurring in 1951 that showed brain damage, but while the cause of death was not determined, a tubercular process was not eliminated. In this monograph alone liver damage has been entirely absent from at least 4 series presented thus far. At this time all 3 drugs must be considered as potentially hepatotoxic. Accurate comparison of side effects probably requires 100,000 patient samples of each drug. The first reporting of jaundice from chlorpromazine only after it had been on the market for 7 months is another example of the need for very large samples.

I should appreciate an explanation from Sainz as to how iproniazid does not allow any pharmacodynamic margin or "psychophysiological homeostasis," while W-1544A does so. For many of us, side effects, dosage adjustment, and lack of therapeutic effects with iproniazid have not been any problem, nor have they been with W-1544A. Possibly, dosage adjustment becomes so automatic after 200 iproniazid-treated cases that selecting different starting doses for individual patients appears to present no problem; for that matter, it has been easier to select the proper initial Marsilid dosage than to decide on the exact dose of the right ataractic needed to accompany it. Because the incidence of minor side effects in my small W-1544A series does seem to be somewhat lower, the already easy decision of Marsilid dosage is even easier with W-1544A.

One last question for Sainz: why did he use only mercurial diuretics for the edema occurring with W-1544A? Robie, in his presentation, mentions Diuril and seems to have had good effects with it; it has seemed to me that relatively small doses of it have been very effective, and that mercurial diuretics and ammonium chloride are required only in cases of very severe edema where Diuril and dosage reduction will not reduce the edema with sufficient rapidity.

Summary

In my series, 243 patients received Marsilid with an over-all improvement rate of 70 per cent and no need for discontinuance of the drug in any case, although one case required dosage reduction to 25 mg./day for edema; the 43 acute schizophrenic patients showed fairly good results. The other 180 cases were primarily agitated depressions; however, some reactive depressions included among them also did very well. In my small series of 40 cases treated with W-1544A, there were no major side effects, and probably a slightly faster action than with iproniazid. Clinically, W-1544A seems to me to be possibly a stronger drug, but this is not sufficiently obvious to consider it 4 times as strong as iproniazid. Both drugs showed recovery time for depressions nearly as fast as that of EST, and both drugs produced the fastest recovery time when used with EST.

Reference

1. Saunders, J. C., N. Radinger & N. S. Kline. 1957. A theoretical and clinical approach to treating depressed and regressed patients with iproniazid. (Report read at 12th Annual Convention of the Society for Biological Psychiatry, June 15 and 16, at Atlantic City, N. J.)

THE PLACE OF IPRONIAZID IN BIOCHEMICAL PSYCHIATRY

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An earlier report on 40 depressed patients (25 females and 15 males, ranging in age from 20 to 78 years) indicated that iproniazid (Marsilid*) elevated mood, improved appetite, increased energy, and brought about a sense of well-being in 37 of the individuals studied.[1] This group of patients seemingly had a greater incidence of favorable response and fewer complications than those reported by other observers.[2] This disparity may be due to different techniques of administration of iproniazid and to different criteria for selection of patients for treatment. Our method of administration of the drug has been comparable to that of digitalization. "Marsilidization" was accomplished with 50 mg. of the substance 3 times a day and subsequently maintained with the minimal effective dose. Iproniazid was added to our armamentarium in July 1957. A summary of continued observations extending over 16½ months is the basis of this report.

The previous study included observations on all patients at the Silver Hill Foundation who received iproniazid. The present study is restricted to data obtained from 45 individuals (30 females, 15 males) cared for exclusively by the author. Sixteen of these patients are continuing from the earlier study.[1] The admitting diagnoses were: manic-depressive (depressed), 16; psychoneurotic depressive reaction, 4; depressive equivalent, 2; involutional depressive reaction, 7; arteriosclerosis with depression, 6; and psychoneuroses (mixed) with depression, 10 (TABLE 1). All of these persons had been treated previously with biochemical stimulants, tranquilizers, and sedatives without significant beneficial effect on their psychiatric illnesses. These patients have been under observation for an average of 2 years and 2 months. The maximum length of time is 8 years, the minimum 1½ months.

The persons studied fall naturally into two groups: those who did and those who did not receive electroconvulsive treatment (ECT). Twenty-two patients received ECT and 23 did not. Results in both groups have been satisfactory (S) or unsatisfactory (U), depending on whether the individual was returned to his predepression adjustment level.

Iproniazid and Electrotherapy

Considering first the group of patients who received ECT (TABLE 2): electrotherapy was not a new experience to 11, since one or more courses (8 to 12 treatments) had been administered to them before iproniazid came into more general use. When depression again recurred, electrotherapy and iproniazid were administered. Three patients received their first physiological treatment as a combination of electrotherapy and iproniazid. Another 4 cases required interim iproniazid to sustain freedom from depression. Iproniazid alone relieved depression in 2; accordingly, no additional electrotherapy was required. The

* Hoffmann-La Roche.

remaining 2 patients of this group received electrotherapy, but not in relation to iproniazid.

A closer scrutiny of individuals in the group that received ECT reveals the following pertinent information. One patient, an 80-year-old arteriosclerotic woman with depression, had recurrent depressive episodes that a course of electrotherapy relieved repeatedly for approximately 30 days. Iproniazid, as a postelectrotherapy supplement, gave the patient 8 months of relief from depression but, unhappily, it became necessary to discontinue iproniazid because

TABLE 1
ADMISSIONS:
DIAGNOSTIC CLASSIFICATIONS

Diagnosis*	ECT group				Non-ECT group			
	Patient No.	Age	Sex	Time observed	Patient No.	Age	Sex	Time observed
M-D (Dep.)	1	73	F	8 yrs., ½ mos.	23	54	F	6 yrs., 7½ mos.
	2	68	F	1 yr., 3½ mos.	24	53	F	1 yr., 4½ mos.
	3	43	M	4 yrs., 10½ mos.	25	64	F	1 yr., 2½ mos.
	4	64	M	7 yrs., 1½ mos.	26	38	F	1 yr., 0 mos.
	5	49	F	5 yrs., 5½ mos.				
	6	77	M	1 yr., 1½ mos.				
	7	57	M	0 yrs., 7½ mos.				
	8	61	M	4 yrs., 5½ mos.				
	9	51	M	1 yr., 9½ mos.				
	10	48	F	4 yrs., 8½ mos.				
	11	64	F	8 yrs., 1½ mos.				
	12	29	F	1 yr., 6½ mos.				
Dep. Reac.	13	68	M	1 yr., 1½ mos.	27	56	F	1 yr., 2½ mos.
	14	54	F	0 yrs., 11½ mos.	28	31	F	0 yrs., 1½ mos.
GAS c̄ Dep.	15	74	F	7 yrs., 3½ mos.	29	61	F	0 yrs., 2½ mos.
	16	80	F	2 yrs., 6½ mos.	30	66	M	2 yrs., 8½ mos.
	17	77	F	1 yr., 8½ mos.				
	18	71	F	0 yrs., 7½ mos.				
Inv. Dep. Reac.	19	57	M	0 yrs., 6½ mos.	31	53	F	0 yrs., 9½ mos.
					32	61	M	2 yrs., 9½ mos.
					33	65	M	0 yrs., 8½ mos.
					34	58	F	0 yrs., 1½ mos.
					35	54	M	0 yrs., 9½ mos.
					36	53	F	0 yrs., 8½ mos.
PN (Mixed) c̄ Dep.	20	57	M	4 yrs., 9½ mos.	37	44	M	0 yrs., 9½ mos.
	21	40	F	0 yrs., 6½ mos.	38	33	F	0 yrs., 9½ mos.
	22	50	F	1 yr., 2½ mos.	39	58	F	0 yrs., 3½ mos.
					40	63	F	0 yrs., 5½ mos.
					41	51	F	0 yrs., 5½ mos.
					42	32	F	1 yr., ½ mos.
					43	44	M	
Dep. Equiv.					44	38	F	0 yrs., 10½ mos.
					45	50	M	0 yrs., 1½ mos.

* Abbreviations: M-D, (Dep.), manic-depressive (depressed); Dep. Reac., depressive reaction; GAS c̄ Dep., generalized arteriosclerosis with depression; Inv. Dep. Reac., involutional depressive reaction; PN (Mixed) c̄ Dep., Psychoneurosis (mixed) with depression; and Dep Equiv., depressive equivalent.

TABLE 2
ECT GROUP

Patient No.	Sex	Age	Diagnosis*	Mos. on Ip.†	Total gm. Ip.	Courses of ECT	Mos. Since ECT	Av. No. ECT's with Ip.	Av. No. ECT's without Ip.	ECT with Ip. Supp.	ECT with Ip. Simul.	Results	Current daily dosage (Mg.)	Comment
6	M	77	M-D (Dep.)	5	4.14	2	4½	4	7	Yes	Yes	S	15	
8	M	61	M-D (Dep.) and Hyp.	4½	4.49	2	8	6	12	Yes	Yes	S	0	Hypertension
9	F	51	M-D (Dep.)	4½	7.19	3	7	6	9	Yes	Yes	S	10	
10	F	48	M-D (Dep.)	6	9.01	5	8	6	10	Yes	Yes	S	0	Congenital syphilis
11	F	64	M-D (Dep.)	10½	9.58	4	9	6	8.5	Yes	Yes	S	25	
21	F	40	PN (Mixed) c̄ Dep.	3½	7.20	4	0	12.5	7.5	Yes	Yes	S	0	
1	F	73	M-D (Dep.)	10	20.27	1	11	0	8	Yes	No	S	50	
16	F	80	GAS c̄ Dep. and Hyp.	7	15.45	3	4	8	10	Yes	Yes	U	0	Syncope
17	F	77	GAS c̄ Dep. and Hyp.	12	20.84	2	19	0	7	Yes	No	S	0	
4	M	64	M-D (Dep.) and Hyp.	11½	21.29	5	15	6	8.5	Yes	Yes	S	0	Coronary; syncope
5	F	49	M-D (Dep.)	3½	10.05	4	1½	4	12	Yes	Yes	U	0	
18	F	71	GAS c̄ Dep.	7	3.76	1	5½	8	0	Yes	Yes	S	5	No hypertension; no alcohol
19	M	57	Inv. Dep. Reac.	2½	10.95	1	4	4	6	Yes	Yes	S	0	
12	F	29	Schizoaff. Dis.	½	2.20	2	5½	8	3	Yes	Yes	U	0	
20	M	57	PN (Mixed) c̄ Dep.	3½	5.29	2	19	0	8	Yes	No	S	15	
3	M	43	M-D (Dep.)	2½	10.35	1	27	0	7	Yes	No	S	0	
15	F	74	GAS c̄ Dep. and Hyp.	14½	27.00	2	15	0	8	Yes	No	S	50	Syncope
7	M	57	M-D (Dep.)	1	1.97	1	7½	0	8	Yes	No	U	0	Improved prostate
14	F	54	Dep. Reac.	5	6.50	1	24	0	5	Yes	No	S	0	Rheumatoid arthritis
2	F	68	M-D (Dep.)	8½	5.62	2	15	0	5	Yes	No	S	15	Dermatitis
13	M	68	Dep. Reac.	1½	1.19	1	7	0	11	Yes	No	U	0	Postural hypotension
22	F	50	Schizo. Paranoid	2	5.60	1	6	0		No	No	U	0	ECT and insulin coma

* See TABLE 1.
† Ip. = iproniazid.

of an increasing vasomotor sensitivity and a resulting severe hypotension. A 68-year-old man with chronic depression had previously benefited from additional electrotherapy, but combined therapy was contraindicated because of the patient's sensitivity to iproniazid; the rapid development of postural hypotension precluded its use even in minute doses. A 29-year-old girl, incorrectly diagnosed as a manic-depressive, benefited neither from electrotherapy nor iproniazid. A 50-year-old woman included in the ECT group proved to be a paranoid schizophrenic with depression who, while taking iproniazid, gained weight and improved physically; however, with improvement in the depressive element in her sickness, she demonstrated more of a paranoid reaction and, ultimately, electrotherapy and insulin coma were required to relieve the illness. Finally, a 57-year-old man had previously received electrotherapy that relieved the overt depression. Gastrointestinal symptoms then emerged, and iproniazid was administered to him, without favorable result, when these symptoms were wishfully but erroneously regarded as evidence of lingering depression.

In summary: of 22 patients only 2, or 10 per cent, with the cardinal symptom of depression did not benefit from iproniazid employed as a complement or as a supplement to electroconvulsive treatment. Ten of 13 patients (77 per cent) with recurrent depression necessitating repeated or sustaining courses of ECT required no more electrotherapy when iproniazid was used in combination with electric treatments. When electrotherapy alone was employed, 9.5 electroconvulsive treatments per series were required; when combined with iproniazid, only 6.5 treatments per course were necessary, a reduction of 31 per cent.

Iproniazid Without Electrotherapy

In those patients who did not receive ECT (TABLE 3), iproniazid alone produced satisfactory results in all but 3. Among the 3 failures 1 was a 53-year-old woman with a mixed psychoneurosis, another was a 32-year-old woman with schizoaffective disease, and the third a 33-year-old woman with anxiety hysteria. Hence, it would seem that iproniazid is less effective in those conditions in which diagnosis is less precise.

Combining the results obtained in the 2 groups, 36 (80 per cent) of the 45 patients seemingly benefited from iproniazid. If the calculations are based on the 40 verified depressions, then 90 per cent obtained satisfactory results.

Complications

In our earlier study[1] postural hypotension, dermatitis, impotence, and dependent edema occurred frequently enough to warrant prudence in iproniazid administration. Subsequently, iproniazid (5, 10, and then 15 mg./day) has been successfully resumed in the woman who had previously reacted with a dermatitis. All patients with a bothersome dependent edema were women and were controlled with acetazolamide (Diamox) until the maintenance dose (ranging from 5 to 33 mg. a day) was established, whereupon the edema became insignificant and diuretics were no longer required. Impotence in the male is no longer a concern. In fact, 2 men with chronic prostatitis and persistent worry over their sexual impotence were apparently more potent. Intractable postural hypotension was the most significant complication encountered in the 45 patients. The person with vascular hypertension appeared to be the most likely to develop persistent orthostatic hypotension with syncope. Repeated clinical and laboratory examinations of the more than 100 patients treated with iproniazid at the Silver Hill Foundation have revealed no hepatic involvement.

Dosage

Of those patients considered to be successfully treated with iproniazid, the total quantity ranged from 0.7 to 27 gm. The duration of iproniazid treatment has been as short as 2 weeks and as long as 15 months. The usual technique of administration continues to be 150 mg. each day until distinct improvement of mood or until serious side effects appear, whereupon gradual reduction of the drug to a maintenance dose is begun. The maintenance dose is established slowly in the range of 5 to 33 mg./day. Abrupt discontinuance of the drug, even though the patient has remained free of depressive signs and symptoms for 2 weeks, may result in a relapse. In the light of our present knowledge, it appears impossible to anticipate accurately the duration of treatment or the minimum maintenance dose—empiricism prevails. Fourteen of the patients for various reasons interrupted iproniazid consumption for 1 week to 4 weeks on 1 or more occasions. A larger dose was not required, nor was iproniazid less effective when reinstated.

TABLE 3
NON-ECT GROUP

Patient No.	Sex	Age	Final diagnosis	Months on Ip.	Total Gm. Ip.	Results	Current daily dosage (Mg.)	Comment
29	F	61	GAS c̄ Dep.	2	4.83	S	25	CVA; hypertension
44	F	38	Dep. Equiv.	8½	9.02	S	10	
26	F	38	M-D (Dep.)	7½	7.81	S	25	
34	F	58	Inv. Dep. Reac.	1½	3.04	S	15	
35	M	54	Inv. Dep. Reac.	½	1.78	S	0	
39	F	58	PN (Mixed) c̄ Dep.	2	3.00	S	20	
25	F	64	M-D (Dep.)	1	5.40	S	0	Rheumatoid arthritis
28	F	31	Dep. Reac.	2	2.50	S	10	
40	F	63	PN (Mixed) c̄ Dep.	5½	1.95	S	10	
23	F	54	M-D (Dep.)	5½	12.75	S	25	
31	F	53	Inv. Dep. Reac.	1½	.70	S	0	
42	F	32	Schizoaff. Disorder	1	2.58	U	0	
37	M	44	PN (Mixed) c̄ Dep.	2½	5.67	S	0	
30	M	66	GAS c̄ Dep.	7	5.03	S	10	CVA; coronary; hypertension
33	M	65	Inv. Dep. Reac.	2	2.73	S	0	
36	F	53	PN (Mixed) c̄ Dep.	½	.57	U	0	
38	F	33	Anxiety Hysteria	1	3.84	U	0	
24	F	53	M-D Dep.	15½	17.73	S	10	Angina pectoris; hypertension
45	M	50	Dep. Equiv.	1½	2.38	S	0	
27	F	56	Dep. Reac.	8	11.64	S	0	
32	M	61	Inv. Dep. Reac.	11	14.36	S	25	Hypertension
41	F	51	PN (Mixed) c̄ Dep.	5	4.06	S	10	
43	M	44	PN (Mixed) c̄ Dep.	12	10.56			

Conclusions

Many psychiatrists find that psychotherapy for depression is successful in psychoneurotically lowered mood, and that relief is often slow. Psychotherapeutic relief of endogenous depression often may coincide with the upward phase of the patient's manic-depressive cycle rather than with insight into his aggressive attitudes.

Iproniazid definitely helps to relieve the symptoms of depression, irrespective of the psychopathology; in our experience, it is more effective than any other chemical in endogenous depressive disorders. Even chronic psychoneuroses manifested by depression, lassitude, low energy, loss of weight, and discouragement are frequently relieved with iproniazid and, when "Marsilidized," such patients are more accessible to psychotherapy. A large portion of the patients (about 75 per cent of the non-ECT group) would have been considered for electrotherapy but for iproniazid. At the Silver Hill Foundation the average daily census is about 50 patients. In 1955, 45 individuals required electroconvulsive therapy; in 1956, 38; and in 1957, 28. The time in residence of depressed patients is decreased, and more are successfully treated as outpatients. Depressions appearing as complications of arteriosclerosis or secondary to it often respond well. Headache, when it is a depressive equivalent, is relieved by iproniazid. Patients suffering from schizophrenic or schizoid personality reactions with depressive features may gain weight and feel better, but no essential alteration in their thinking pattern occurs; ultimately they may become worse.

The apprehension that iproniazid potentiates barbiturates during their simultaneous administration has faded. Before the establishment of an iproniazid maintenance dose, meprobamate or barbiturates are often indicated to alleviate the insomnia of the depressed patient. Alcohol is contraindicated, since iproniazid provides a built-in relief for hang-over, thus inviting repeated overindulgence; alcohol plus iproniazid produces quick inebriety and increases the risk of syncope from orthostatic hypotension.

It has been observed that depressed patients with low-grade infections such as chronic prostatitis or arthritis are sometimes partially relieved of the pain and discomfort while taking iproniazid. The low-back pain and the tendency to premature ejaculation of patients with chronic prostatitis are diminished. Nocturnal angina pectoris, accompanying generalized arteriosclerosis with coronary artery disease, is decreased in frequency and intensity.

Iproniazid and electrotherapy seem to be synergistic. When used concomitantly, the number of required electric treatments is decreased. Patients in our group in 1955–1956 required an average of 10.96 treatments per course; in 1956–1957, 9.57; and, since the advent of iproniazid in 1957, it has decreased to 7.8. If the physician reserves electrotherapy for severe depression and employs iproniazid as a complement or a supplement to electrotherapy, it is possible for 90 per cent of the patients to maintain their regained psychic equilibrium.

In psychiatric office practice iproniazid has made possible the treatment of a significant number of depressed individuals as outpatients rather than in residence. The availability of such a useful drug has changed the psychiatrist's attitude. No longer must he wait helplessly until the end of the cycle in manic-depressives for whom electrotherapy is contraindicated; no longer must he rely only on sedation or electrotherapy to make the senile and arteriosclerotically depressed patient more comfortable.

We cannot confirm reports that agitation accompanying some depression may be made worse by iproniazid; however, because the agitatedly depressed patient is so frequently suicidal, we utilize electroconvulsive therapy promptly.

In the psychotherapeutically refractory depressive reactions and tenacious endogenous depressions iproniazid often shortens the duration of the illness. Thus, in iproniazid we may have a chemical as important to biochemical therapy of depressive disorders as penicillin is in bacterial infections. Neither is effective in all cases, but both increase the probabilities of relief. No other known chemical substance has thus far proved as effective as iproniazid in relieving the miserable symptom of depression.

References

1. Scanlon, W. G. & W. M. White. 1958. Iproniazid (Marsilid): Its use in the office treatment of depression. Am. J. Psychiat. **114:** 1036.
2. Ayd, F. J., Jr. 1957. A preliminary report on Marsilid. Am. J. Psychiat. **114:** 459.

Discussion of the Paper

David C. English (*Mercywood Neuropsychiatric Hospital, Ann Arbor, Mich.*)*:* Although I certainly agree with every major point Scanlon has made, several were especially impressive.

Scanlon mentioned that the patients he summarized in the present study included only those whom he had followed himself. This is both unusual and excellent, since there are still too many subjective factors involved both in making a diagnosis and in judging improvement. No behavior chart as yet can accurately reflect the total picture of a patient's improvement, and such behavior charts necessarily depend on subjective impressions of whoever does the rating.

Scanlon mentioned that other clinicians had reported more (and even prohibitive) side effects, while he himself had noticed very few, if any, and none that could not be easily managed. At least 18 clinical studies with iproniazid (Marsilid) have been reported in this monograph. Of the 18 opinions, no more than 3 could be considered distinctly unfavorable to the use of MAO inhibitors because of side effects. In his presentation Robie explained part of the difference in results as due to some so-called "side effects" being residuals of the illness itself: the initial symptoms had subsided, but not yet entirely disappeared. Both depressed and schizophrenic patients, without any drugs, frequently complain of dizziness, tension, fatigue and lassitude, insomnia, "funny feelings in my head," "jumpiness," and impotence or frigidity. These symptoms, of course, are not due to iproniazid, but disappear completely if the patient makes a 100 per cent recovery. Even without complete recovery the residual symptoms in the majority of patients are much reduced compared with the complaints on admission.

Robie's second pertinent comment was that some of the symptoms displayed by patients while taking iproniazid were due to pyridoxine (vitamin B_6) deficiency. Some of this deficiency was undoubtedly due to iproniazid, but a considerable proportion resulted from poor nutrition, which is characteristic of a depressed or schizophrenic patient. This deficiency responds completely to administration of a multivitamin preparation with a high vitamin B_6 content. Since adjuvant vitamin medication is indicated temporarily in all such patients because of their poor nutrition, such vitamin B_6 deficiencies cannot be considered a side effect in the sense of the rarely occurring liver damage.

The only possible conclusion is that 90 per cent of those using iproniazid find only occasional minor side effects, which can be managed, as did Scanlon, without reducing the drug. Although it cannot be denied that severe liver damage has occurred with Marsilid, its over-all incidence is certainly less than 1:10,000. As Kline reports elsewhere in this monograph, the usually accepted incidence of electroshock treatment (EST) complications is 1:1000, that is, more than 10 times that of Marsilid. Serious side effects, no matter how rare, naturally receive more attention than the good results in thousands of patients, still these complications always must be kept in mind, even though there is 1 chance in 12,000 that they may occur. An analogous case is that of the phenothiazines. The clinician has to watch for possible blood dyscrasias as well as liver damage, even when using newer derivatives that as yet have not produced such complications.

A very rarely mentioned but extremely useful point that Scanlon made was that iproniazid and EST are mutually complementary, so that the need for EST was cut 90 per cent and the number of treatments from 9½ to 6½. The use of such combinations is relatively rare in research studies, since the only way to be absolutely sure of the degree of the effectiveness of a drug is to give it alone. However, in working with private patients, who form the bulk of both Scanlon's work and my own, treatment must always be directed toward improving the patient's illness as rapidly as possible even when this means utilizing simultaneously 2 different forms of treatment and, therefore, making such treatment valueless for inclusion in a research study. At Mercywood Hospital I have been able to confirm Scanlon's observations on the efficacy of combined treatments. One hundred and eighty seven patients treated with EST alone required an average of 8.7 EST, while 67 patients with agitated depressions of an equivalent degree of severity, who received both EST and iproniazid, required only 5.4 EST, a 38 per cent reduction.

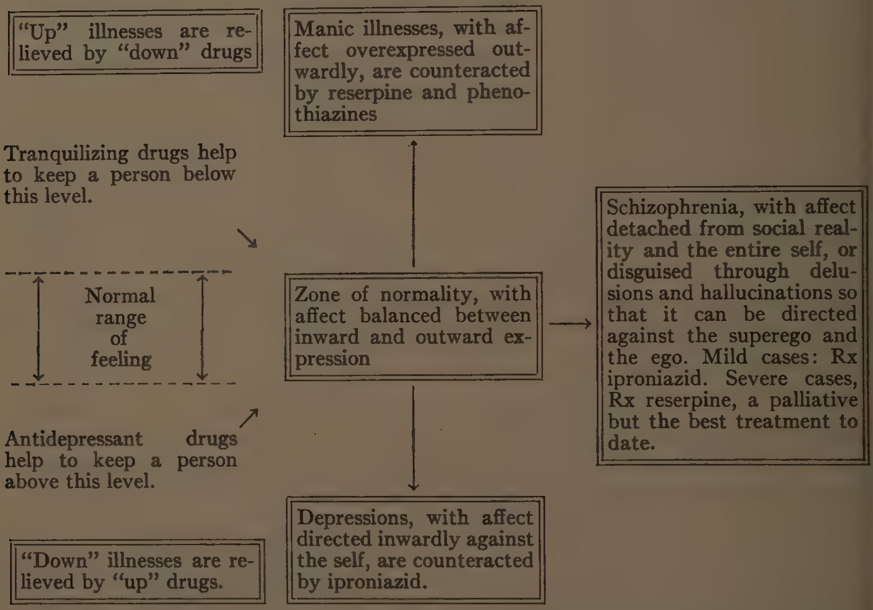
As mentioned above, such combination methods are rarely reported, especially in psychiatry. An analogous example from neurology is the testing of a new anticonvulsant, alone, in patients who are refractory to previous anticonvulsants alone or in combination. Since the established drugs often work best when used together, it is surprising that a new drug can work sufficiently well alone while competing with established drugs in combination. Once such a drug has become established, it is immediately used in combinations, especially by private practitioners who are trying to help individual patients rather than to build up research series. These combinations almost certainly provide better results than the new drug alone.

Only a few schizophrenics are included in Scanlon's series. It is obvious from the papers presented in this monograph that the results in schizophrenics have varied tremendously, about one third reporting good results, one third no significant change, and the remainder reporting changes for the worse. Both of these contradictory results may be easily explained, and good therapeutic effects produced in all but the most deteriorated of schizophrenics if iproniazid is balanced with tranquilizing drugs, usually phenothiazines. In work with 43 acute illnesses (overtly of one-year or less duration), a good result occurred in 70 per cent and no change in the remaining 30 per cent.

Elsewhere in this monograph Kline states that iproniazid always produced a change in 100 per cent of the schizophrenics, but in one third or more the change was for the worse. This occurs because iproniazid increases the patient's overt social activity and outgoing behavior and, since schizophrenics have much more fragile ego boundaries than the normal person, this change can very easily lead to antisocial activities: for example, when a severely ill catatonic schizophrenic reaction is converted into a catatonic excitement state several windows may be broken. FIGURE 1 shows an admittedly oversimplified diagram worked out after a patient literally did break windows. In other words, the patient's ego boundaries were exceeded, and when mepazine (Pacatal), used because of its absence of "flattening" effects, was added, the patient remained socially communicative, but her behavior became socially proper. This "balancing" must be done not only individually, but often 3 times a week, since a schizophrenic's ego strength does not return to normal as quickly as in depression. Obviously, the more acute the illness, the better the response. There is admittedly a narrow area between overstimulation and overtranquilization of a schizophrenic. However, where the ultimate prognosis appears to be fairly good, especially with private patients who are seen with sufficient frequency to make possible adjustment of the drug when required, this method has helped. Occasionally, a lower-than-usual maintenance dose of iproniazid would accomplish the same thing; however, in more than one half of the patients in whom this has been attempted it resulted only in a reversion to their initial social withdrawal.

It has frequently been stated that new drugs are valuable both in treatment and in sharpening of diagnostic categories. The appended FIGURE 1, if it does not help the diagnosis of schizophrenia, is interesting because it again shows that schizophrenia is the unknown illness. Although the ego boundaries of manics or depressives are in many ways stronger than those of schizophrenics, yet a severely ill patient on either end of this continuum certainly shows entirely asocial behavior. However, most depressed patients do respond to iproniazid, which elevates them, and many manics respond to tranquilizing drugs that depress them to the middle of the continuum. Schizophrenia continues to be the unknown illness; it certainly does not fit in this continuum; in fact, some cases respond best to tranquilizers, some best to antidepressants, and some only to both. Schizophrenic reactions, then, show characteristics of both manic and depressed illnesses but, as everyone realizes, these reactions differ entirely from either illness. The unique characteristic of schizophrenic reactions is not even hinted at in this diagram. Rather, its value lies only in showing that the balancing of both drugs helps certain patients who will not respond to either one. It also shows that the schizophrenic reaction, although showing characteristics of both illnesses, is yet entirely separate and basically unrelated to either.

The diagnostic categories that have already been separated by the use of iproniazid are those of "reactive depression" as opposed to "endogenous depression." Although exact definitions of these 2 terms are impossible, as ordinarily used, the former equals a "neurotic depressive reaction" while the latter is a "psychotic depressive reaction." The terms mild and severe would probably work just as well for the two classifications, since some patients who appear profoundly depressed, and who formerly could improve only with EST, show excellent contact with reality. In the same way, the original meaning of "reactive depression" was probably adapted from "traumatic neurosis" (as used



"Up" illnesses are relieved by "down" drugs

Manic illnesses, with affect overexpressed outwardly, are counteracted by reserpine and phenothiazines

Tranquilizing drugs help to keep a person below this level.

Schizophrenia, with affect detached from social reality and the entire self, or disguised through delusions and hallucinations so that it can be directed against the superego and the ego. Mild cases: Rx iproniazid. Severe cases, Rx reserpine, a palliative but the best treatment to date.

Zone of normality, with affect balanced between inward and outward expression

Antidepressant drugs help to keep a person above this level.

"Down" illnesses are relieved by "up" drugs.

Depressions, with affect directed inwardly against the self, are counteracted by iproniazid.

FIGURE 1. This chart is unique in that it connects illnesses that appear to be totally unrelated and offers an easy method for the determination of treatment. Iproniazid effectively relieved 84 per cent of 180 cases of acute agitated depression in 28 days, as compared with 92 per cent of 842 cases in 22 days with Reiter electrostimulations. Forty-three cases that received the iproniazid analogue phenelzine did equally well. Both drugs showed only mild and easily controlled side effects. Pacatal gave immediate relief of initial agitation and balanced possible overstimulation by iproniazid, itself produced some euphoria, and showed no "flattening" action. Pacatal with iproniazid did not show the universally found symptomatic exacerbation produced in one third of the apathetic patients by iproniazid alone, because the Pacatal balanced the iproniazid energizing to keep all of the patients within their weakened ego boundaries, so that two thirds of them were much improved. Iproniazid provides the most accurate appraisal of apathetic ego defenses and sharpens the differential diagnosis within depressions by separating mental and nervous illnesses. These two classifications, or endogenous and reactive depressions, form opposite ends of a continuous line upon which Deprol relieves only mild neurotic depression, which is an illness close to neurasthenia. Although manic and depressive illnesses form opposite poles of a straight line, with normality between them, each illness being relieved by one of the pharmacological antagonists, reserpine and iproniazid, schizophrenia, which is helped by combinations of both drugs and which fits into the continuum at right angles to the others, is still the "unknown illness" as far as its ultimate cause is concerned. The nonaddictive iproniazid, when administered with electrostimulation, reduces the amount of EST needed by one third.

This report is taken from the first published systematic study in which antidepressants and tranquilizing drugs were combined from the beginning. It is also the largest antidepressant series in private hospitals in the United States and one of the largest series in the United States regardless of type of hospital.[1]

by Freud) to indicate a sudden illness in a relatively stable individual and apparently a direct result of a sudden severe and disabling environmental factor.

Although much of the time it is true that the premorbid personality of a psychotic depressive shows indications of the illness to follow, yet it is also true that there are many cases in which this cannot be seen even if one is looking for it, knowing that the patient is psychotic. Some of the discrepancies obtained with iproniazid occur because of the admixture of "neurotic" or "dysphoric" reactions. These respond best to tranquilizing drugs and improve with benactyzine-meprobamate (Deprol). Since such patients are actually more neurasthenic than depressed, the use of iproniazid, although stimulating them to more activity, only produces more tension and anxiety. The "depression" of which the layman speaks is usually a neurotic one, and shows excellent results with benactyzine-meprobamate. A direct comparison of this drug with Pacatal in 40 cases each, showed equal results; Pacatal probably induces slightly more rapid improvement than other phenothiazines because of the euphoriant action which occurs in about 25 per cent of patients. However, the use of either Deprol or Pacatal in definite psychotic depressions produced about 38 per cent improvement at best, as compared with over 70 per cent for iproniazid.

The balancing of iproniazid and tranquilizers mentioned above is even more effective in agitated depressions since the patients are extremely hyperkinetic even before iproniazid is given. I found excellent results in 128 agitated depressions, using Marsilid with Pacatal in approximately equal doses, milligram for milligram. In a series of only 15 patients who seemed to be on the borderline between neurotic and psychotic depressives, I observed even faster results with Deprol and Marsilid together. The oversedation that occurs initially with the meprobamate was partially counteracted by iproniazid and the possible later overstimulation from iproniazid was likewise counteracted by the Deprol. The improvement time with this group of patients was shortest of any. The reasons for this are difficult to evaluate. It is difficult to believe that Deprol speeds the action of Marsilid, since a certain period of time is required for inhibition of monoamine oxidase. A group of borderline patients such as this group is obviously impossible to duplicate. The reason Deprol was used instead of Pacatal was that the former would not be sufficiently strong to sedate an actual psychotic but, theoretically, should be effective in a milder illness. Probably the only conclusion that can be drawn from this simultaneous use of Marsilid with Deprol is that cases that appear to be borderline can be treated for both reactive and endogenous depressions at the same time, the undesired immediate effects of each drug counteracting the other.

Although the basic psychodynamics are obviously not changed by antidepressants, the clinical depression is relieved. Analysts themselves agree that a depression is a direct contraindication to psychoanalysis. Elsewhere in these pages, Kline points out that psychoenergetics is the last and neglected third of Freud's psychopathology. It certainly must be true that this third is definitely changed. Although recovery from a depression simply prepares a person for exploratory interviews, such a patient feels relief from tension and depression: symptoms that usually initiate psychotherapeutic interviews.

It has recently been mentioned that serum transaminase levels are more effective for detecting incipient liver damage than the standard liver battery. The one patient in our experience with definite Marsilid intolerance at 75 mg. per day developed upper gastric upsets about 4 days before her liver-function test began rising. Accordingly, several transaminase determinations were made in order to rule out a coronary occlusion; since these were completely within normal limits, they did not help to alert us to the liver damage.

Reference

English, D. 1959. Marsilid and Pacatal in the treatment of depressive schizophrenic reactions. Mich. State Med. Soc. J. **58**: 205–211.

IPRONIAZID: A BRIEF REVIEW OF ITS INTRODUCTION AND CLINICAL USE

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Shortly before the synthesis of iproniazid (Marsilid*) and its testing at Sea View Hospital, Staten Island, N. Y., in 1951, I concluded a lecture on streptomycin at the New York Academy of Medicine with a statement that, although this material represented a great advance over previous therapy in tuberculosis, some additional drug must be developed to eliminate or at least control the tremendously destructive lesions of osseous tuberculosis. I stated that younger generations of physicians might then remark: "What were these lesions and why the intense interest in bone and joint tuberculosis so many years ago?" We had no conception that a new drug of such potency would be available within only a few months.

I have written fully about my experimental use of this drug, the benefits noted, the toxicities encountered and controlled, the translation of its benefits and its toxicities to use in conditions other than tuberculosis of bones and joints, and undesirable lay publicity and the resultant damage.[1-9] The appended bibliography will furnish the reader a ready reference to the clinical facts reported. It is impossible to repeat all of this material in a brief introduction to the subject. Therefore, I propose merely to outline past clinical investigation, findings, and conclusions. A review of all patients treated with Marsilid by us will be undertaken shortly. Since this will cover a period of eight and one-half years, an adequate number of the patients will come within the follow-up group, many with more than five years of observation. Certain further positive conclusions not previously appreciable can be based upon this group. Although much knowledge from this long-range study is, of course, at hand, it will not be specifically outlined here except for the tempering of conclusions gained from our previous investigations.

History

In 1951, at Sea View Hospital, Robitzek *et al.*[10, 11] began the experimental use of three new antituberculous chemicals. Two of these, isoniazid (Rimifon*) and iproniazid were shown to be of value and have been continued in use. In the fall of that year iproniazid had been shown to have certain toxicities in excess of those exhibited by isoniazid. Despite this, the use of iproniazid was continued, and it became recognized that it had much greater value than isoniazid in the treatment of bone and joint lesions. In spite of initial adversities, it was demonstrated that it could be used safely. Not only were toxic side effects controlled, but the beneficial effects exhibited in the osseous-tuberculous cases were transferred to use in other lesions. Three times, due to injudicious, undesired, and disastrous publicity, iproniazid has almost been removed from our medical armamentarium, but each time its usefulness has been so demonstrated and documented that it has been retained. It is probable that, although other drugs may be developed to supplant it for certain usages, it will continue to have a definite, assigned place in therapy.

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* Hoffmann-La Roche.

Dosage

This drug should never be released except under a physician's prescription and continued observation. The assignment to the physician of direct responsibility for its use will assure care in its employment. The dosage varies considerably, depending upon the indication. I estimate the maximum dosage at 4 mg./kg. body weight/day and suggest that it be given at 8-hour intervals to maintain continuity of therapeutic levels in the blood serum. This dosage should be limited to such potentially disastrous conditions as severe tuberculous infections of bones and joints and similar involvements. Maintenance of such dosage should be restricted to a relatively short period, such as 1 or 2 weeks.

The usual safe dosage for moderately severe conditions in which it is employed would appear to be 2 mg./kg. body weight. Note that even here we are considering potentially disastrous situations, such as infection and lesions of the mesoderm (tumors) causing pain, that can be relieved satisfactorily only by narcotics. This is quite apart from the wide usage of the drug in psychiatry or general medicine. In the above-mentioned dosage, and for specific reasons, we have continued many patients for from 3 to 5 years and more without any difficulties. This may be true for us as orthopedic surgeons, treating lesions closely watched while under our care, and entirely false where the drug is employed for more general medical purposes.

From our observation and in consequence of our special interest, we know that 1 mg. or even 0.5 mg./kg./day can be entirely adequate in certain situations. We have seen an emaciated gastrectomy patient gain and maintain weight, with dosage raised, lowered, and finally balanced at ½ mg./kg./day, and we have seen euphoria maintained at ¼ mg./kg./day.

It is of greatest importance that the drug be used for a specific indication in which it has been shown to be effective and within a reasonable dosage level. Coupled with this is the need for continuous, knowledgeable medical observation. When the drug is thus employed, it has proved to be so valuable that it is difficult to imagine any other medication as a potential rival, unless it is so clearly innocuous as to permit careless use: a quality not inherent in iproniazid. As in the case of any drug, there will always be an occasional patient who cannot tolerate its use at any dosage level.

Indications

The beneficial effects on tuberculous patients quickly became apparent. The drug was not merely accepted as nearly specific for patients with bone and joint tuberculosis with sinuses, but its various individual beneficial actions and side effects were tabulated. As an orthopedic surgeon I took no blood pressure readings and, for that reason, I did not note what was later found, namely, depression of blood pressure. Since others have investigated this action and reported on it, I shall not discuss it further.

The beneficial effects noted were: development of euphoria, relief of pain, overcoming anorexia, weight gain in debilitated patients, temperature reduction, wound healing without regard to organisms present, marked control of tuberculous infection in bones and joints, and decalcification of osseous structure without weakness.

In addition to the above, there seem to be other little-understood changes that can be only vaguely expressed. These were metabolic change, fibrositic effect, and collagen alteration.

Granted that the last three are not scientifically established as to action, method of motivation, or result, on a clinical basis they are well established. As a clinician rather than a scientist, I must leave these to be evaluated and studied by others; however, it has not been unusual for a clinician to be in advance of the scientist. One need only consider such drugs as opium and digitalis to realize that frequently, if one always waited for the scientist to give comfort to the patient, unconscionable delay in the relief of human ailments would frequently be the consequence. I have not learned all of the answers to the questions that iproniazid raises, but further inquiry should expand its control and usefulness.

Iproniazid should be employed for the above indications rather than for specific clinical entities. A few specific clinical entities, previously uncontrolled by other means, appear to be susceptible to this drug. Tuberculous lesions of bones and joints have responded so well that no other medication is now being used at Sea View, St. Luke's, Polyclinic, or other hospitals in the city of New York that we serve. In acute osteomyelitic infections, where organisms resistant to antibiotics are encountered, the drug is tremendously useful in controlling temperature, promoting euphoria, causing weight gain, and establishing wound healing without regard to the bacteria present. The healing of indolent wounds, such as burns, has been greatly stimulated. Depressed states have been shown susceptible to control (these are best left to the psychiatrist). Weight gain has frequently been achieved in the emaciated, regardless of the cause of the condition.

The drug has been shown to be almost a specific for acute lupus erythematosus. Decalcification noted to occur in osseous structures has been employed to demonstrate sequestra, leading to their removal. Aside from the surgical aspects mentioned, a selective review of the medical literature on this drug, in addition to the personal bibliography appended, is indispensable to the medical practitioner. Particularly important is the use of iproniazid in the relief of pain and as an adjuvant in metastatic lesions, especially when mesodermal elements are involved.

This short review can be no more than a brief outline. None of the bibliographical material, documented on a factual clinical basis, has required retraction. Intensive laboratory studies of the patients reported were done. Despite the claims of others, no actual change was found in such laboratory findings as those on urine or blood, circulating eosinophils, or 17-ketosteroids, except for a minor reduction of the platelet count. I could discover no reduction of the red blood cell count or hemoglobin. One should not be disturbed by the occurrence of psychogenic phenomena, since these are always reversible on reduction or discontinuance of the drug; reports of toxic effects on the liver must be examined for factual data and for proof that these were specifically the result of iproniazid and did not arise from other causes.

When Robitzek *et al.* and I deny severe liver toxicity in the dosages advocated, we challenge those of an opposite opinion to document thoroughly any such instances. No drug, not even aspirin, is completely innocuous. The good must be balanced against the bad. In iproniazid we have a medication that must not be removed from use.

References

1. Bosworth, D. M. 1956. The treatment of tuberculous lesions of bones and joints with iproniazid (Marsilid). New York. J. Med. **56:** 1281–1284.
2. Bosworth, D. M. 1958. Bone and joint tuberculosis in childhood. J. Bone and Joint Surg. To be published.
3. Bosworth, D. M., J. W. Fielding, L. M. Demarest & M. Bonaquist. 1955. Toxicity to iproniazid (Marsilid) as it affects osseous tuberculosis. Quart. Bull. Sea View Hosp. **16:** 134–140.
4. Bosworth, D. M., J. W. Fielding, H. J. Wilson, M. G. Acosta & L. M. Demarest. 1955. A comparison of the efficacy of iproniazid (Marsilid) and isoniazid (Rimifon) in the treatment of bone and joint tuberculosis. Quart. Bull. Sea View Hosp. **16:** 125–133.
5. Bosworth, D. M., J. W. Fielding, M. G. Acosta & L. M. Demarest. 1955. Use of iproniazid in nontuberculous bone and joint infections. J. Am. Med. Assoc. **157:** 132–136.
6. Bosworth, D. M., H. A. Wright & J. W. Fielding. 1952. The treatment of bone and joint tuberculosis: effect of 1-isonicotinyl-2-isopropylhydrazine. A preliminary report. J. Bone and Joint Surg. **34-A**(4)**:** 761–771.
7. Bosworth, D. M., H. A. Wright, J. W. Fielding & H. J. Wilson. 1953. The use of iproniazid in the treatment of bone and joint tuberculosis. **35-A**(3)**:** 577–589.
8. Bosworth, D. M., H. A. Wright & J. W. Fielding. 1952. Marsilid in the treatment of tuberculous orthopedic lesions. Quart. Bull. Sea View Hosp. **13:** 52–73.
9. Bosworth, D. M. 1958. Discussion of the history of Marsilid. J. Clin Exptl. Psychopathol. **19:** 11–26.
10. Robitzek, E. H., I. J. Selikoff & G. G. Ornstein. 1952. Chemotherapy of human tuberculosis with hydrazine derivatives of isonicotinic acid. Preliminary report of representative cases. Quart. Bull. Sea View Hosp. **13:** 27–51.
11. Selikoff, I. J., E. H. Robitzek & G. G. Ornstein. 1952. Toxicity of hydrazine derivatives of isonicotinic acid in the chemotherapy of human tuberculosis. Preliminary report. Quart. Bull. Sea View Hosp. **13:** 17–26.

Discussion of the Paper

B. L. Reynolds, J. B. Codington, A. R. Mansberger, Jr., and R. W. Buxton, (*Department of Surgery, University of Maryland School of Medicine Baltimore, Md.*): The chemistry laboratory has recently become the site of widespread investigative efforts by general surgeons attempting to define more clearly the sequential stages in wound repair. Chemical phases have been described by producing circular or elliptical wounds in experimental animals and using biopsy technique. After establishing the wound, we and others have shown that an exudative phase is followed by an interval in which mucopolysaccharide elements predominate; this stage blends with a period in which mucoprotein elements, particularly collagen, aggregate.[1] We have described the phenomena of contraction, migration of regenerate, and epitheliazation, and their response to one additive coenzyme.[2] Considerable disagreement exists on the relative importance of these phases, particularly that of the mucopolysaccharide interval. Many investigators feel that the presence of hexosamine in the polysaccharide phase represents tissue debris and extravasation of serum and plasma protein incidental to the repair process. Bosworth has observed a considerable clinical response after administration of iproniazid (Marsilid) in

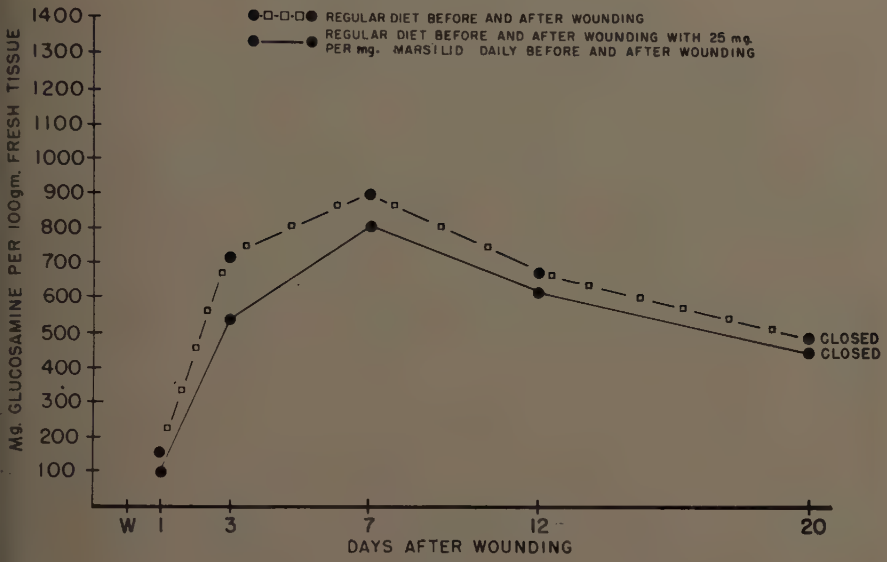

FIGURE 1

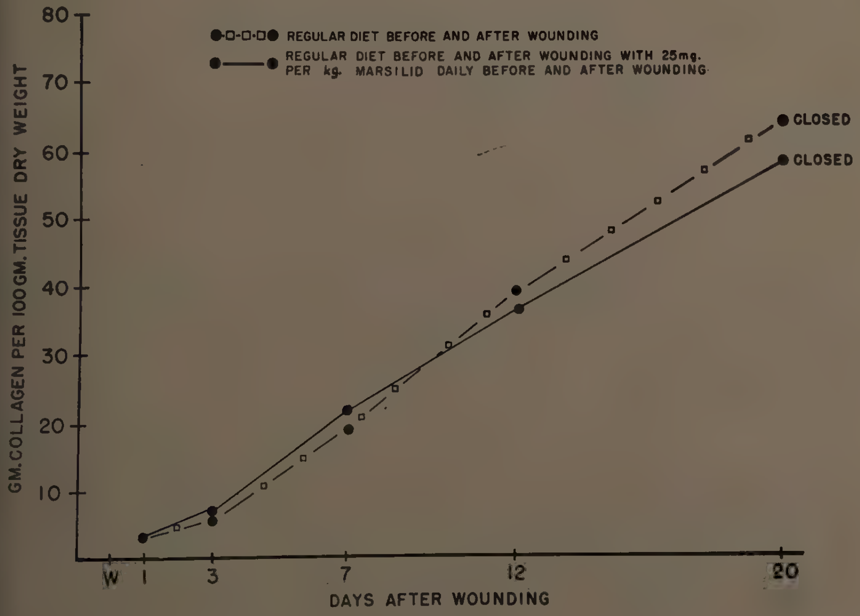

FIGURE 2

tuberculous patients with persistently draining sinus tracts and foci of infection. It seemed possible to us that monoamine oxidase inhibition might influence hexosamine and polysaccharide levels in the second interval of chemical repair. Accordingly, we undertook to evaluate this possibility.

Circular wounds were produced in albino rats in a segment of skin with considerable mobility. One group was used as controls and was administered a balanced diet and tap water. A second group received, in addition, 25 mg./kg. of Marsilid intramuscularly in a contralateral extremity. On representative days a sampling was carried out by removal of biopsy specimens from the wound center (or migratory regenerate) for morphological and chemical analysis. The most important of the latter were determinations of hexosamine, collagen, and lactic acid.

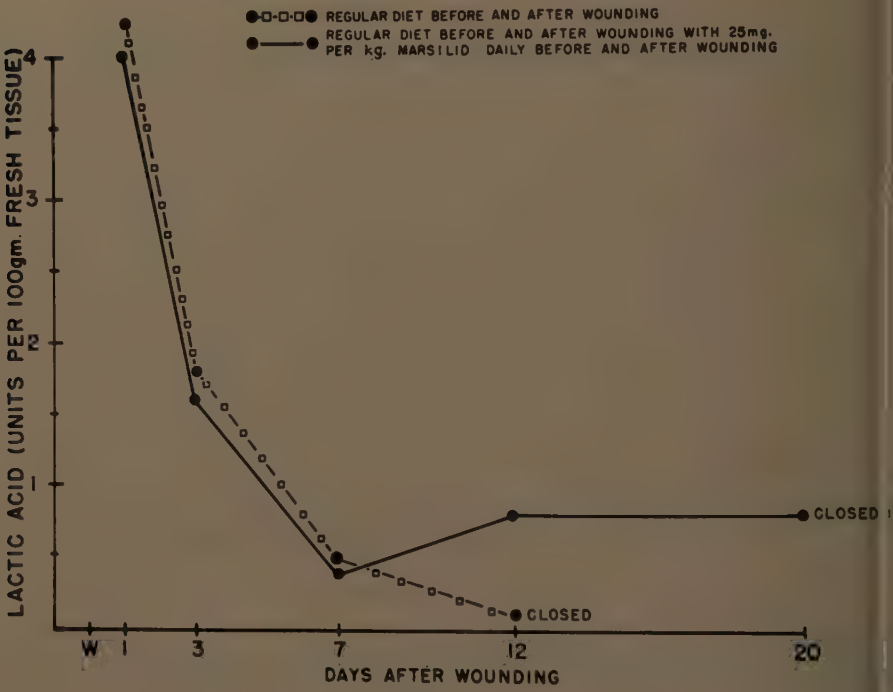

Figure 3

Results

The hexosamine content indicated by FIGURE 1 suggested earlier utilization of this material by Marsilid-treated animals. FIGURE 2 suggests that formation of collagen was neither augmented nor delayed by Marsilid. Most interesting is the result of lactic acid titrations (FIGURE 3). Between the third and fifth or seventh days lactic acid values fell a little more rapidly in the Marsilid-treated animals than in controls, but rose to higher levels thereafter.

Interpretation

If the fall in lactic acid is significant, and if it is taken as an index of return to a more nearly oxidative substrate, the administration of this monoamine oxidase inhibitor is active in the mucopolysaccharide phase of repair. The lower levels of hexosamine, which may well indicate more rapid utilization of this agent, tend to support this observation. Moreover, a comparison of FIGURES 4 and 5 and of FIGURES 6 and 7 (which are photomicrographs of morphological sections on the third and tenth postwounding days) shows that the cell content, maturation, and organization of the migratory regenerate are more advanced in the polysaccharide and the mucoprotein phase of the Marsilid-treated animal. Although there is no chemically measurable increase in total collagen or a more rapid collection of this protein in the Marsilid-treated animal, the appearance of closely packed regenerate suggests a repair element with a greater tensile strength by the tenth postwounding day. Collagen content must have been influenced by the drug dosage administered these animals who, by the tenth postwounding day, had received 15 injections of 25 mg./day. This dosage level was continued until either the animals died or the twentieth postwounding day had been attained. Beyond the tenth day all animals lost weight and exhibited periodic convulsive seizures and marked exophthalmos. Subsequent work by others has shown that the activity of iproniazid continues for several days beyond the last administered dose. We feel that a large element of toxicity has altered interpretation beyond postwounding day 10. In our opinion, positive activity has been grossly demonstrated between wounding and postwounding day 10 as a result of Marsilid injections; however, additional studies are required. The effects of protein depletion, corticoid stimulation, and oxidative uncoupling agents along with Marsilid administration are the subjects of separate reviews.

Acknowledgment

The Marsilid used in these studies was generously supplied by Hoffmann-La Roche Inc., Nutley, N. J.

References

1. DUNPHY, J. E. & K. N. UDUPA. 1955. Chemical and histochemical sequences in the normal healing of wounds. New Engl. J. Med. **253:** 847.
2. REYNOLDS, B. L., J. B. CODINGTON & R. W. BUXTON. 1958. Wound healing, a study of the response of injured tissues to the co-enzyme adenosine 5-monophosphate. Surgery. **44:** 33.

[FIGURES 4–7 on pages 816–819]

FIGURE 4. Photomicrograph of morphological section on third postwounding day of a Marsilid-treated animal. Compare with FIGURE 5. Hematoxylin and eosin. ×50.

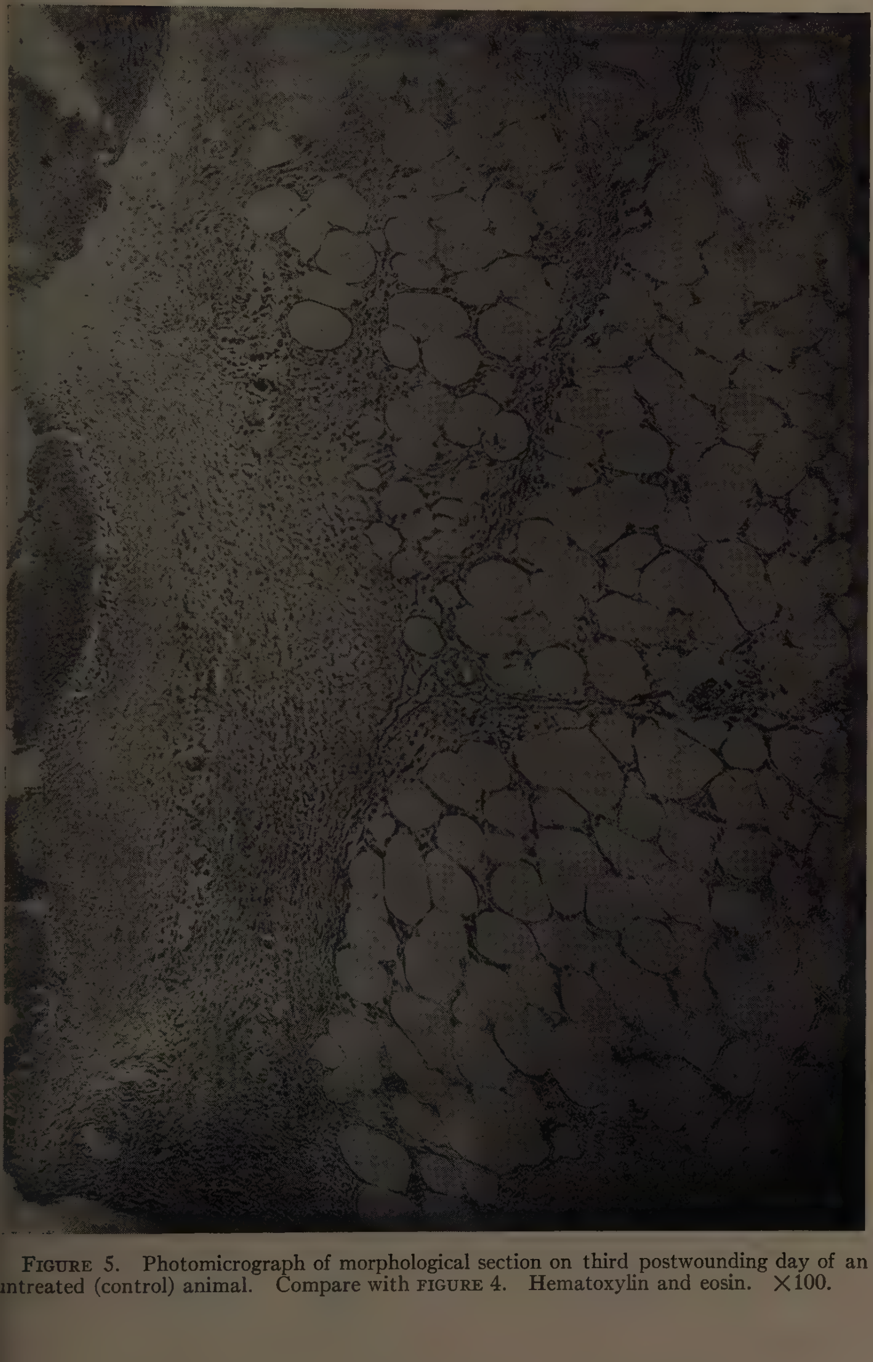

FIGURE 5. Photomicrograph of morphological section on third postwounding day of an untreated (control) animal. Compare with FIGURE 4. Hematoxylin and eosin. ×100.

FIGURE 6. Photomicrograph of morphological section on tenth postwounding day of a Marsilid-treated animal. Compare with FIGURE 7. Hematoxylin and eosin. ×100.

FIGURE 7. Photomicrograph of morphological section on tenth postwounding day of an untreated (control) animal. Compare with FIGURE 6. Hematoxylin and eosin. ×50.

THE EFFECTS OF IPRONIAZID AND OTHER AMINE OXIDASE INHIBITORS IN RHEUMATOID ARTHRITIS

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Many of the central nervous system manifestations characteristic of rheumatoid arthritis are favorably altered by the administration of small doses of iproniazid (Marsilid*). These characteristic manifestations include retarded psychomotor activity, intolerance to stressful situations, vasomotor reactions, transient bouts of flushing, decreased tolerance to cold, transient paresthesias, muscular weakness, and alteration in deep-tendon reflexes. With larger doses there is lessening of the inflammatory joint manifestations.

Clinical Response to Iproniazid

The pharmacological action of iproniazid is complicated, and many systems of the body may be affected simultaneously. In patients with rheumatoid arthritis one of the most important clinical effects of iproniazid is stimulation of the central nervous system. This effect is thought to be related to an increase of serotonin and norepinephrine in the brain,[1] which results from amine oxidase inhibition. Increased psychomotor activity usually appears rapidly when only small doses of the compound are administered and is accompanied by increased strength, appetite, and ability to concentrate. Often, the degree of psychomotor retardation existing before treatment is not recognized until after therapy is instituted.

Iproniazid also may produce a sympatholytic effect on the autonomic nervous system. In general, this varies with dosage and duration of treatment and the condition for which the compound is administered. Usually the degree of sympatholytic activity varies with the dose of the drug. This effect of iproniazid becomes obvious in diseases associated with dysfunction of the autonomic nervous system. Early in the treatment of patients with rheumatoid arthritis there are a lessening of palmar perspiration and vasomotor reactions and an increased tolerance to a cold environment. Small doses of iproniazid have little influence on objective joint manifestations; when larger doses of the drug are administered, inflammatory joint manifestations diminish. It is not known whether this is due to sympatholytic activity or another pharmacological action. When joint manifestations improve, there is increased sympatholytic activity, manifested by dryness of the mouth, blurring of vision, postural hypotension, constipation, urinary hesitancy, and diminished ability to ejaculate. There is an associated increased pain threshold and also muscular fasciculations, hyperreflexia, and clonus. Bilateral or unilateral pitting edema of the upper or lower extremities may occur rarely. When the compound is discontinued, these effects slowly subside and, simultaneously, the pre-existing joint manifestations return. A similar effect is observed in patients with acute neuroreflex dystrophy. Loosening of the skin and subcutaneous tissue occurs to a limited degree in patients with systemic sclerosis. In the upper extremities

* Hoffmann-La Roche.

this is noticeable in the forearms and hands, but little change is noted in the digits. When Raynaud's phenomenon is present, there are fewer color changes of the fingers following emotional stress or exposure to a cool environment. It is well known that postural hypotension occurs that may be uncontrolled when larger doses of the compound are administered. It is possible that this effect of iproniazid is important in rheumatoid arthritis and related diseases.

Recently Zbinden *et al.*[2] reported that decreased levels of 5-hydroxytryptamine found in guinea pig intestine following the administration of reserpine can be blocked by pretreatment with iproniazid. Granules in enterochromaffin cells made visible by histochemical technique disappear following reserpine, but remain visible in pretreated animals, suggesting that iproniazid protects the 5-hydroxytryptamine in the enterochromaffin granules. We have reported that patients with rheumatoid arthritis manifest an exaggerated sensitivity to extravascular administration of serotonin and histamine.[3] It is possible, although not proved, that the mast cell is responsible for the formation of these amines or related substances and that delayed inactivation results in the characteristic mesenchymal reaction of rheumatoid arthritis. Lessening of the mesenchymal reaction that usually occurs when large doses of iproniazid are administered may be due to a blocking action exerted on mast cells, which is unrelated to amine oxidase inhibition.

We have studied the excretion rate of serotonin metabolites in a patient with active rheumatoid arthritis following the intravenous administration of 2 mg. of 5-hydroxytryptamine-β-C^{14}creatinine sulfate containing 4 μc. of activity; a comparison was made of these rates and those of 2 normal control subjects (FIGURE 1).[4] Before treatment approximately 10 per cent of the administered radioactive serotonin was excreted within 5 hours. After treatment for 1 month with 20 mg. daily of 1-benzyl-2-(5-methyl-3-isoxazolylcarbonyl) hydrazine, coded as RO 5-0831/1, an analogue of iproniazid, 85 per cent of the radioactive serotonin was excreted within the same period (5 hours). However, despite the increased excretion of serotonin metabolites, the ratio of 5-hydroxyindole acetic acid to other metabolites remained the same within a 24-hour period before and after treatment, suggesting that monoamine oxidase was not completely inhibited or that some mechanism of action other than monoamine oxidase inhibition was responsible for this effect.

It is our opinion that alterations occur within the liver that are not demonstrable by the tests presently used to measure hepatic function. We have observed gynecomastia in males and diminished tolerance to alcohol during the administration of iproniazid despite normal findings on studies of hepatic function (including transaminase determinations). In addition, the action of numerous unrelated compounds is potentiated; these include general and local anesthetic agents, barbiturates, corticosteroids, ganglionic blocking agents, morphine, derivatives of atropine, and the 4-aminoquinoline compounds.

Under certain circumstances iproniazid stimulates the healing of tissues. It has been reported that iproniazid administered orally hastens the healing of tuberculous sinuses.[5] When applied locally, in the form of a 3 to 5 per cent ointment, it stimulates the healing of trophic ulcers associated with Raynaud's phenomenon or systemic lupus erythematosus (FIGURE 2). No effect is manifest when this ointment is applied to ischemic ulcers associated with arteriosclerosis obliterans, to varicose ulcers, or to decubitus ulcers.[6] The introduction of aqueous iproniazid into an actively inflamed rheumatoid joint has no demonstrable immediate effect on the inflammatory reaction.

Administration of Iproniazid

Iproniazid and related drugs should be used only by physicians familiar with their pharmacological action. The dosage is variable and depends upon the effect desired from the drug and the disease for which it is given.

In general, patients with rheumatoid arthritis and related diseases respond to small doses of iproniazid more than do others for whom the drug has been recommended. Nevertheless, it is important to remember that there is often wide variation in individual drug requirements. Initially, there is a latent period varying from a few days to a few weeks, during which clinical response to the drug may not appear. After clinical improvement does appear there is a variable cumulative effect, and the dosage must be reduced accordingly before subsequent effects of overdosage appear. The early manifestations of overdosage may not be recognized unless the patient is questioned specifically regarding insomnia, dryness of the mouth, constipation, urinary hesitancy, inability to ejaculate, lightheadedness, muscular fasciculations, postural hypotension, hyperreflexia, and clonus. The appearance of one or more of these side effects indicates that the drug is beginning to accumulate within the body and that continuation of the dosage may eventually lead to toxicity. It is

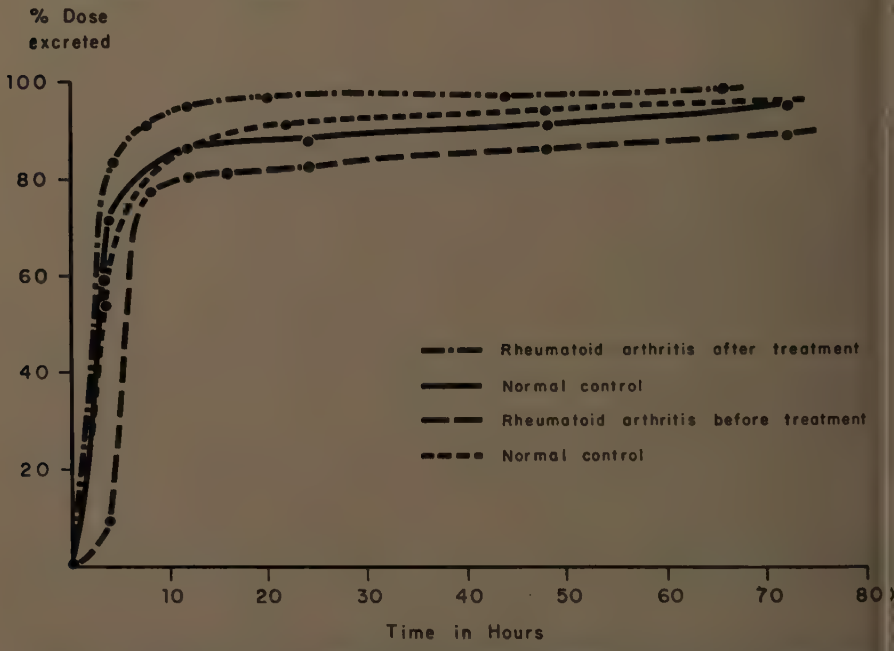

FIGURE 1. Excretion of radioactivity after dose of C^{14}-serotonin before and after treatment of rheumatoid arthritis. A comparison with normal control subjects is also shown.

important to realize that toxic reactions are likely to persist for weeks, despite reduction of dosage or discontinuation of the drug. Prolonged toxic reactions are more frequent when the initial dosage of iproniazid or a related analogue is large.

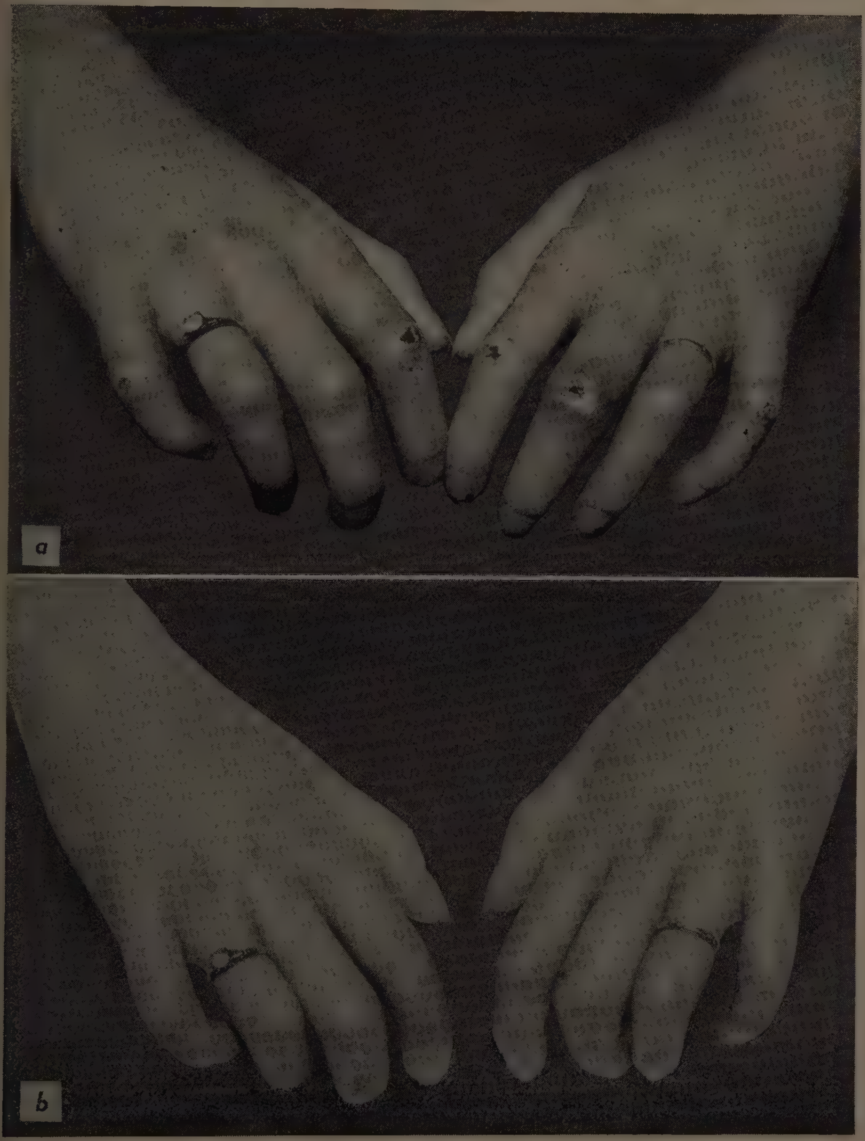

FIGURE 2. (*a*) Painful trophic ulcerations over the proximal interphalangeal joints of a 24-year-old woman who had severe progressive systemic sclerosis. (*b*) Complete healing of ulcers 19 days after 3 per cent iproniazid ointment was applied twice daily. Pain diminished within 24 hours after the ointment was applied.

Toxic Reactions

In 1952, when we began to use iproniazid, it was administered in doses of 150 mg. daily to 30 patients with rheumatoid arthritis. Excessive stimulation of the central nervous system appeared between the second week and the third month of therapy. When the dose was reduced to 50 mg. daily or less, the initial response to the drug was variable and often associated with a longer latent period.

In 5 of the 30 patients who received 150 mg. of iproniazid daily, marked postural hypotension, constipation, exaggerated tendon reflexes, and generalized clonus occurred before the fourth month of therapy. In 2 patients, clonus persisted for 3 days and hyperreflexia for 3 weeks after iproniazid was discontinued. In 3 of the 5 patients, temporary impairment of hepatic function, evidenced by diminished pseudocholinesterase and increased retention of sulfobromophthalein, was observed. Laboratory tests showed normal reactions 2 or 3 weeks after the medication was stopped. Other laboratory tests, including serum bilirubin and alkaline phosphatase values, were not altered. No further impairment of hepatic function was observed in these patients when iproniazid was readministered in doses of 25 to 50 mg. daily 3 months later. The cumulative action of iproniazid was obvious after 6 months of treatment in 10 of 67 patients with rheumatoid arthritis who were given 50 mg. daily. Postural hypotension, constipation, and hyperreflexia developed as accumulation occurred. None of these 10 patients showed alteration in hepatic function, as measured by diminution of pseudocholinesterase activity or retention of sulfobromophthalein. Even after further reduction in dosage to 25 mg. daily or less, a cumulative effect was still noted and, in 2 of these 10 patients, it persisted after the dosage was reduced to 10 mg. daily. Eventually it was found that increased psychomotor activity was maintained in these 2 patients with 5 mg. of iproniazid daily.

Hepatocellular Lesions Related to Iproniazid

One of the most serious complications related to the administration of iproniazid is hepatocellular damage. During the first 18 months that we studied both iproniazid and isoniazid, jaundice occurred in 3 patients of 227 who received either compound as the sole therapeutic agent. Two patients of 108 who received 300 mg. of isoniazid became jaundiced after 2 months and 11 months, respectively. One patient, a 57-year-old man, had pre-existing biliary cirrhosis that was proved by biopsy at the time of a cholecystectomy prior to therapy; the other patient, a 52-year-old man, had pre-existing mild portal cirrhosis proved by biospy of the liver before therapy was started.[7] Jaundice occurred in a 26-year-old woman after the administration of 150 mg. of iproniazid daily for 2 months. The results of hepatic function tests were compatible with hepatocellular damage. The total serum bilirubin during the course of her illness varied between 1.4 and 2.4 mg. per 100 ml.; alkaline phosphatase was 6 Bodansky units, cephalin flocculation was 3 plus, and the thymol turbidity was 8 units. All three patients recovered uneventfully within 4 weeks after the onset of jaundice.

Because these hepatic complications were encountered, the dosage of iproniazid was subsequently reduced to 50 mg. daily or less and the drug was used only as a temporary adjunct in a balanced program of therapy for patients with persistently active or progressive rheumatoid arthritis. In contrast to 3 patients (of the initial 227) in whom jaundice developed after the daily administration of either 300 mg. of isoniazid or of 150 mg. of iproniazid, not a single case of complicating jaundice was observed in 921 patients administered iproniazid in doses of 50 mg. daily or less for periods varying from a few months to 5 years.

Possible Factors Responsible for Hepatocellular Damage

Iproniazid is a derivative of hydrazine, well known for its hepatotoxic action, although derivatives may be considerably less toxic. Studies of normal human subjects have revealed that about 50 per cent of iproniazid is excreted in the urine in 24 hours and 70 per cent in 48 hours.[8] This finding suggests to us that long-term administration of iproniazid results in accumulation not only of the drug, but of metabolites that may be responsible for hepatocellular damage unless dosage is carefully adjusted for each individual.

The inhibition of oxidizing enzymes within the liver may result in the accumulation of substances that may act as toxins within hepatic cells, resulting in inflammation or hepatic necrosis. The rapidity with which the substances accumulate and cause this complication may depend upon the disease for which iproniazid is administered.

The cumulative effect of iproniazid occurs more commonly than is suspected. In our patient, hepatocellular alterations occurred only when overdosage and excessive accumulation of the drug had occurred. When the initial dosage of iproniazid was low and the maintenance dosage adjusted to the individual patient, accumulation, overdosage, and hepatocellular damage were not manifest.

The microscopic changes that occur in the liver after the administration of iproniazid resemble those of viral hepatitis or hepatic-cell necrosis.[9] A pre-existing virus within the liver conceivably could cause acute viral hepatitis if the administration of one of the hydrazide derivatives weakened hepatic defense mechanisms in some manner.

The possibilities mentioned are entirely conjectural, and the cause for hepatocellular damage is not known. The lack of hepatocellular damage in 921 of our patients has not been explained. At the present time we believe that dosage is the most important although not the sole factor responsible for the hepatocellular complication. In addition to the use of a low initial dosage of iproniazid, we have administered the drug once daily rather than in divided dosages. This may decrease the time necessary for the liver to detoxify an offending metabolite or to correct a metabolic alteration that might otherwise result in hepatic damage.

In the majority of patients to whom we administered iproniazid, pre-existing alterations of the plasma proteins, characterized by elevations in the alpha-2 or gamma fractions or both and by diminished albumin, were present. Alterations of the plasma proteins also occur in patients with tuberculosis. This phenomenon may be significant since the incidence of hepatitis has been low in these patients despite the large doses of iproniazid administered. Thus, preexisting alterations in hepatic function resulting in increased alpha-2 or gamma globulin may prevent or lessen the severity of hepatocellular damage. Koelle has suggested that a difference in pharmacological interactions with iproniazid and other drugs may occur because of adsorption of iproniazid to plasma proteins, which removes the drugs from the field of activity.[10]

In many of our patients iproniazid has been administered simultaneously with chloroquine phosphate or hydroxychloroquine sulfate.[11] It is possible that the strong affinity of these 4-aminoquinoline compounds for hepatic cells prevents hepatic damage in some unexplained manner. Some of our patients also received small daily doses of a corticosteroid (usually 3 to 7.5 mg. daily of prednisolone) while iproniazid was administered; the corticosteroids may protect the liver.

Observations on the Effect of Other Amine Oxidase Inhibitors

During 1958 we investigated a number of amine oxidase inhibitors and compared their pharmacological effects with those of iproniazid.

RO 2-6797 (2-ethyl-benz [*f*] isoindoline hydrobromide) is a relatively weak amine oxidase inhibitor in comparison to iproniazid. It, also, blocks the peripheral effects of serotonin as measured in dogs.[12] This drug was given to 16 patients in dosages varying from 50 to 100 mg. for periods of from 2 weeks to 5 months. Ten patients had rheumatoid arthritis, 5 had either involutional or senile depression, and 1 had extensive metastatic malignancy. Elevation of mood was noted in 10 of the 16 patients. In each instance only mild psychomotor retardation was present before therapy. No effect was noted in 5 patients with involutional or senile depression. Nervousness occurred in 5 patients after the second week of therapy; decrease in appetite occurred in 4 patients; and anorexia developed in 3; postural hypotension was not observed. In 3 patients there was a slight rise in blood pressure (averaging 20 mm. Hg systolic and 9 mm. Hg diastolic pressure) while the drug was administered; pressures returned to previous levels when medication was stopped. Seven of the 10 patients with rheumatoid arthritis stated that less joint pain was present while medication was administered, although no significant effect on objective joint manifestations was observed. One patient with metastatic malignancy, who required narcotic drugs in large doses every 1 to 2 hours, noted remarkable relief from pain and required only about one fourth as much pain-relieving medication. It is concluded that this compound has an amphetaminelike action that results in mild elevation of mood, that it appears to potentiate narcotics and sedatives, but that it is not as effective as iproniazid and has no particular advantage over other compounds presently available.

RO 4-1018 is the isopropylhydrazide of mercaptobutyric acid, DL-1(*N*-acetylmethionyl)-2-isopropylhydrazide, and is an amine oxidase inhibitor with an effect similar to that of iproniazid. The drug was administered to 12 patients in doses varying from 100 to 150 mg. daily. Six patients had rheumatoid arthritis and 4 had involutional or senile depressions. Increased psychomotor activity appeared in all patients with rheumatoid arthritis. Postural hypotension, constipation, and insomnia developed in 7 of the 12 patients. It is concluded that this compound has no advantage over iproniazid and that all the effects of overdosage similar to those occurring from overdosage of iproniazid may occur.

RO 5-0700 (2-benzyl-1-picolinylhydrazine) is an analogue of iproniazid. We believe it to be approximately 3 times as effective as iproniazid in stimulating psychomotor activity. It was administered to 15 patients for periods of from 2 to 6 months in doses of from 5 to 40 mg. daily. Eight patients had rheumatoid arthritis or related disorders, 2 had involutional depression, and 5 had moderately severe essential hypertension. Elevation of mood appeared quickly when 30 mg. or more of the compound was administered daily. Psychomotor stimulation was clear by the third day, but effects of overdosage appeared more frequently than with iproniazid. Postural hypotension occurred in 3 patients without hypertension by the end of the second week, and 2 patients with hypertension showed moderate declines in blood pressure by the fourth week of therapy, during which they were receiving 30 mg. daily. Constipation appeared in 9 patients, and hyperreflexia was present in 11 others after the fourth week of therapy. One patient with persistent blood pressure readings of from 220/140 to 210/132 mm. Hg experienced a gradual decline in pressure to 160/96 mm. Hg, 4 weeks after treatment was started. She has received maintenance therapy of 15 to 20 mg. daily for 6 months. It is concluded that this compound is more potent that iproniazid and acts more quickly, although overdosage causes a greater number of undesirable effects. It has a greater effect on blood pressure than iproniazid, is more unpredictable, and the dosage is more difficult to control.

Two of the most potent and effective compounds we have studied are (1-methyl-2-phenyl)-ethyl hydrazine hydrochloride, coded as JB-516, and 1-benzyl-2-(5-ethyl-3-isoxazolylcarbonyl) hydrazine, coded as RO 5-0831/1. Both compounds are considerably more potent than iproniazid. In addition to stimulating psychomotor activity in patients with rheumatoid arthritis, there is a more significant effect on objective joint manifestations than is usually observed with iproniazid when the latter is administered in doses that we consider safe. A mild and consistent effect on the blood pressure was noted in these patients. Improvement began by the second or third week, as manifested by elevation of mood, improved ability to concentrate, increased strength, lessening of musculoskeletal pain, and decrease in joint swelling and tenderness. One instance of edema of both legs and 2 instances of edema of 1 leg have occurred with RO 5-0831/1; the edema subsided spontaneously when the medication was stopped. Marked postural hypotension has occurred in 4 of 30 patients receiving JB-516 and in 1 of 25 patients receiving RO 5-0831/1; however, mild or moderate reductions in blood pressure were observed in almost all patients. Side effects related to overdosage persisted longer in patients receiving JB-516 or RO 5-0831/1 than in those receiving iproniazid. In 1 patient given JB-516, lightheadedness persisted for 2 weeks after the medication was stopped. One patient receiving JB-516 developed hyperreflexia that persisted for 13 days after the drug was stopped. These patients had received 12.5 mg. daily for 2 to 3 weeks before the effect of overdosage appeared. Since then we have limited dosage of this compound in patients with rheumatoid arthritis to 12.5 mg. daily for 3 days; dosage is then reduced to 6 mg. daily in most instances. While the drug is more effective than iproniazid, it is important to remember that toxicity may persist for much longer periods than is usual with iproniazid. The dose of RO 5-0831/1 in patients with rheumatoid arthritis has been 30 mg. daily in 3 divided doses for 7 to 10 days; it is then reduced to 20 mg. daily for from 4 to 6 weeks, when a further reduction in dosage is usually necessary. These dosage schedules have resulted in no serious side actions or hepatic damage, although further observation and study are necessary before conclusions can be made regarding either of these compounds. It is believed that these drugs are capable of causing all the effects that have been observed with iproniazid; therefore, strict regulation of dosage is imperative.

At the present time we have administered JB-516 to 55 patients, 30 of whom had rheumatoid arthritis and 25 of whom had depressive reactions. Another group of 47 patients, consisting of 31 with rheumatoid arthritis, 6 with chronic ulcerative colitis, and 10 with depressive reactions, have received RO 5-0831/1 for periods of as long as 4 months. It is emphasized that the cumulative action of both compounds is greater than that of iproniazid, and overdosage must be avoided by frequent observation of the patients and reduction in medication when indicated.

Discussion

It is clear that neurohumoral dysfunction is a frequent occurrence in various diseases, but it is doubtful that a single biochemical alteration is responsible for the various central nervous system manifestations. The biochemical alterations resulting from the administration of iproniazid and related compounds produce various effects in many diseases. It has been suggested that serotonin may provide the body with a chemical buffering system.[13] It is becoming increasingly clear that neurohumoral dysfunction occurs commonly in various diseases, but whether these changes are primary or secondary manifestations is yet to be determined. Unfortunately, the compounds currently available produce effects throughout the body, and methods of selectively altering their pharmacological action must be found. Because of their narrow chemotherapeutic index, their cumulative action, and their potentiating effect, these compounds must be used only by physicians who are familiar with their pharmacological actions and with the characteristic responses to the drugs. Continuous observation of the patient and adjustment of the dosage is carried out periodically. If satisfactory response to treatment does not occur within a reasonable time, another form of treatment should be substituted.

We do not believe that any disease should be treated with these compounds if dosages large enough to produce serious side effects are required. The most objectionable of these side effects are insomnia of increasing severity, marked constipation, urinary hesitancy, uncontrolled postural hypotension, and marked clonus. Side effects and toxic reactions are most likely to occur when large doses of these compounds are administered for relief of depression, angina pectoris, and progressive essential hypertension. Therefore, we do not believe that these conditions should be treated with the compounds currently available. Alcoholics or patients with pre-existing disease of the liver should not receive these drugs. While it is realized that dosage is not the only factor, it is believed that undesirable side effects and serious toxic reactions can be virtually eliminated by administering these drugs in smaller dosage.

Summary

Rheumatoid arthritis is characterized by an unexplained, diffuse mesenchymal reaction and by a variety of central nervous system manifestations.

Central nervous system manifestations are alleviated by the administration of small doses of iproniazid (an amine oxidase inhibitor) or a related analogue. When larger doses are administered the mesenchymal reaction diminishes.

Iproniazid improves the psychomotor state clinically, inhibits sympathetic activity, and potentiates the actions of certain drugs. Experimental evidence reveals that the amounts of serotonin and norepinephrine in the brain are increased as a result of amine oxidase inhibition, and that the liberation of serotonin from peripheral tissues (induced by reserpine) is inhibited.

Iproniazid or related compounds should be administered only by physicians familiar with their pharmacological actions. Characteristics common to all the compounds investigated include a delayed onset of action, a cumulative effect, and prolongation of toxicity after withdrawal of the drug. The most serious complication of treatment is hepatocellular damage, which appears to be related to overdosage.

Other analogues of iproniazid are being investigated; (1-methyl-2-phenyl)-ethyl hydrazine hydrochloride, coded as JB-516, and 1-benzyl-2-(5-methyl-3-isoxazolylcarbonyl) hydrazine, coded as RO 5-0831/1, appear more effective than iproniazid. With these drugs, central nervous system manifestations subside rapidly and there is greater inhibition of the peripheral mesenchymal reaction. However, further investigation is necessary before the newer analogues can be recommended as therapeutic agents in rheumatoid disease.

Acknowledgment

We acknowledge our appreciation of the supplies of RO 2-6797, RO 4-1018, RO 5-0700, and RO 5-0831 supplied by Hoffmann-La Roche Inc., Nutley, N. J., and of JB-516, supplied by Lakeside Laboratories, Inc., Milwaukee, Wis.

References

1. Shore, P. A. 1958. Possible mechanism of antidepressant action of Marsilid. J. Clin. Exptl. Psychopathol. **19**(2): 56–60.
2. Zbinden, G., A. Pletscher & A. Studer. 1957. Inhibition of reserpine-induced release of 5-hydroxytryptamine in the enterochromaffine system by isopropyl isonicotinic acid hydrazide. Klin. Wochschr. **11**: 565–567.
3. Scherbel, A. L. & J. W. Harrison. 1958. The effect of serotonin antagonists on the exaggerated response to serotonin in patients with rheumatoid arthritis and related diseases. Clin. Research. 6(4): 401–402.
4. McIsaac, W. M. 1958. Presented at the Cleveland Acad. Med. Soc. Exptl. Biol. Med. (November). Cleveland, Ohio.
5. Bosworth, D. M. 1956. Treatment of tuberculous lesions of bones and joints with iproniazid (Marsilid). New York State J. Med. **56**(8): 1281–1284.
6. Scherbel, A. L., G. H. Curtis & J. W. Harrison. 1958. Effect of iproniazid ointment on trophic ulcers associated with Raynaud's phenomenon; clinical note. Cleveland Clin. Quart. **25**(2): 92–94.
7. Scherbel, A. L. 1957. III. The effect of isoniazid and of iproniazid in patients with rheumatoid arthritis. Cleveland Clin. Quart. **24**(2): 90–97.

8. De Ritter, E., L. Drekter, J. Scheiner & S. H. Rubin. 1952. Urinary excretion of hydrazine derivatives of isonicotinic acid in normal humans. Proc. Soc. Exptl. Biol. Med. **79:** 654–658.
9. Zimmerman, H. J. 1958. Some preliminary observations on liver function as related to therapy with iproniazid. Symposium sponsored by Hoffmann-La Roche Inc., The Biochemical and Clinical Aspects of Marsilid and Other Monoamine Oxidase Inhibitors. Chicago, Ill.
10. Koelle, G. B. 1958. Pharmacologic significance of inhibition of monoamine oxidase. J. Clin. Exptl. Psychopathol. **19**(2): 37–44.
11. Scherbel, A. L., J. W. Harrison & M. Atdjian. 1958. Further observations on the use of 4-aminoquinoline compounds in patients with rheumatoid arthritis or related diseases. Cleveland Clin. Quart. **25**(2): 95–111.
12. Randall, L. O. 1958. Personal communication.
13. Page, I. H. 1958. Serotonin (5-hydroxytryptamine); the last four years. Physiol. Revs. **38**(2): 277–325.

Discussion of the Paper

Harry E. Banghart (*Germantown Hospital, Philadelphia, Pa.*): Iproniazid has been investigated for nineteen months. This investigation has taken three directions: the clinical evaluation of the drug in the management of some connective-tissue disorders, an appraisal of its effect on some chemical elements of the blood in patients without bone or rheumatologic disease, and some observations of its action on the bones of pigeons.

Table 1
Marsilid Therapy in Collagen Diseases

Diagnosis	Number of cases	Number of cases with 50 per cent or more subjective improvement
Rheumatoid arthritis	81	52
Rheumatoid spondylitis	16	15
Systemic lupus erythematosus	4	4
Dermatomyositis	10	5
Scleroderma	3	2
Polyarteritis nodosa	1	0
Collagen-vascular disease	3	1

One hundred and eighteen patients suffering with various collagen diseases have been treated with iproniazid. The method of study was identical to that reported in 1958,[1] and the method of evaluation also was unchanged. Results were based on subjective improvement reflected in a heightened sense of well-being, increased strength, greater tolerance of pain, and diminished stiffness. A reduced requirement for steroids also was noted in some cases.

In the previous report[1] the attempt was to evaluate the effect of iproniazid on certain objective findings in rheumatoid arthritis. Changes in redness and swelling of the joints, in skin and muscles, and the degree of mobility and tenderness were quantitated. Again, these were noted, but will not be reported.

The laboratory evidence of improvement in any of the collagen diseases was again conspicuous by its absence. Blood counts, sedimentation rates, electrophoretic patterns, or serologic reactions of these diseases showed no changes that could be attributed to iproniazid.

The results of the use of iproniazid are listed in table 1. Of the 118 patients treated, 81 were suffering with rheumatoid arthritis and 52 of these showed 50 per cent or greater degree of subjective improvement. Rheumatoid spondylitis occurred in 16 cases, and 15 of these showed marked relief of their symptoms.

Four patients with mild subsiding systemic lupus erythematosus were treated with iproniazid; all of these showed marked improvement. The drug was effective in relieving the symptoms of 5 of 10 cases of dermatomyositis. In these cases, response was slow and less complete than in the previous cases.

Improvement occurred within two weeks in two of three cases of scleroderma. The single case of polyarteritis nodosa was not benefited, and only one of three cases of collagen vascular disease was improved.

TABLE 2

EFFECT OF 150 MG. OF IPRONIAZID PER DAY ON CA, PO_4, AND ALKALINE PHOSPHATASE LEVELS

	Control	14 days	28 days
Calcium	9.7*	9.8	9.9
Phosphate	4.0*	3.9	7.7
Alkaline phosphatase	3.0†	5.5	28.9

* Milligrams per cent.
† Bodansky units.

TABLE 3

EFFECT OF IPRONIAZID AND ACTH ON HYDROXYPROLINE AND CALCIUM IN PIGEON FEMUR*

	Hydroxyproline	Collagen† (estimated)	Percentages collagen	Calcium	Percentages calcium
Control	1.16	8.28	100	4.98	100
ACTH‡	1.06	7.56	91	4.60	92
Iproniazid§	1.33	9.49	114	5.56	111
ACTH and iproniazid	1.05	7.49	90	4.57	91

* Each group tested consisted of 10 to 12 (400 to 450 gm.) adult pigeons treated for 20 days.
† Hydroxyproline × 7.14.
‡ Dose: 2.5 U. per 100 gm.
§ Dose: 5.0 mg./kg.

Iproniazid has been administered to nine patients with osteoporosis that had not been relieved by the usual regimens, such as estrogen-androgens and diet. Six of these patients noted definite lessening of pain, improved mental outlook, better appetite, and greater strength and mobility.

In view of these subjective changes in osteoporosis, one patient hospitalized because of nonunion of fractures and severe osteoporosis was treated with 150 mg. of iproniazid daily. The effect of this on the serum calcium, phosphorus, and alkaline phosphatase is shown in TABLE 2. Iproniazid did not affect the calcium, the phosphorus was increased, and the alkaline phosphatase rose from a control value of 3 to 28.9 Bodansky units. Unfortunately, the toxic symptoms of the drug necessitated stopping this study, deterring further investigation at this dosage.

Lucy Wunderly and I then administered iproniazid in a dose of 50 mg./day to 12 individuals who had no evidence of rheumatic, hepatic, or metabolic bone disease. The serum calcium, phosphorus, and alkaline phosphatase levels were determined, before treatment, every 2 weeks after therapy was instituted and after 12 weeks of therapy. There was no significant change in any of the substances determined.

The final study to be reported is of the effect of iproniazid and ACTH on the hydroxyproline and calcium content of pigeon bones. This work was done in collaboration with Louise Feng and Arthur Wase. We have previously shown that ACTH reduces both the total protein nitrogen and the hydroxyproline content of pigeon bones and that, while an androgen effectively counteracts the antianabolic or the catabolic effect (or both) of ACTH on the noncollagenous protein, it has no effect on the collagen as expressed by the hydroxyproline.[2]

Iproniazid appears to increase the hydroxyproline (TABLE 3) in the untreated (with ACTH) pigeon femur, but it does not reverse the ACTH effect. This same effect is noted in the calcium content of these bones; the significance of these findings is still obscure.

References

1. BANGHART, H. E. 1958. The effect of Marsilid on the symptoms of some of the rheumatic diseases. J. Clin. Exptl. Psychopathol. 19(2): Suppl. 1. 114.
2. BANGHART, H. E., L. FENG & A. W. WASE. 1958. Effect of growth hormone, androgen and corticotrophin on bone marrow. Federation Proc. 17: 185.

JOHN MARKS (*Department of Radiotherapeutics, University of Cambridge, Cambridge, England*): In discussing Scherbel's excellent paper I propose to utilize the results from two English pilot trials of monoamine oxidase inhibitors in rheumatic disorders.

The first of these studies, undertaken primarily in rheumatoid arthritis, was conducted at the Charterhouse Rheumatism Clinic in London, England.[1] The second trial, confined to cases of ankylosing spondylitis, I have conducted in Cambridge.

The first study was made on twenty-nine patients: 25 with rheumatoid arthritis, 3 with osteoarthritis, and 1 with ankylosing spondylitis. All these patients had the following major features of their symptomatology: a severe chronic disease with excessive weight loss, muscle wasting, and depression.

At the start of the study, doses of 50 to 100 mg. of iproniazid (Marsilid) per day were tried, but as these did not prove effective, all subsequent treatment was begun at 150 mg. per day. The dose was later reduced as improvement occurred, and maintenance therapy was usually achieved with less than 150 mg. per day. No other medication was given at the same time, apart from aspirin or similar mild analgesics.

The following results were found (TABLE 1). In 12 patients the treatment was stopped within 2 weeks because of side effects, most of which were minor and could have been ignored; nearly all were seen in the early stages of the trial, and discretion led to withdrawal of the drug.

The remaining 17 patients were under treatment for from 2 to 12 months and all showed improvement, although 2 have since stopped treatment because of side effects, 1 patient suffering a serious collapse. No cases of jaundice were seen in this series.

Improvement manifested itself by: (1) relief of depression (with mood elevation), (2) a gain in weight (in nearly all the patients), and (3) improved functional capabilities. The majority of patients were convinced that their functional capacity had been improved, and it was possible to demonstrate this by the grip test in about 60 per cent. The improved functional capacity, however, may not represent any change in the underlying pathology, but may be due to mood elevation and a greater pain tolerance. In fact, it would appear that, in selected patients with rheumatoid arthritis, iproniazid plays its part as adjuvant therapy rather than by an effect on the actual disease process.

My own studies have been undertaken in cases of ankylosing spondylitis.

Thus far twenty-eight patients have been treated with amine oxidase inhibitors. The patients were essentially unselected, except that all showed severe, chronic, ankylosing spondylitis based on irrefutable clinical and radiological evidence. Both the "burnt-out" type and active cases have been treated. The majority, before commencement of treatment, were depressed, had loss of weight, and considerable functional disability.

TABLE 1
IPRONIAZID IN RHEUMATOID ARTHRITIS

Total treated	29
Stopped within 2 weeks	12
Treated 2 to 12 months	17
Improved	17

TABLE 2
IPRONIAZID IN ANKYLOSING SPONDYLITIS

Total treated	16
Improved	14
Weight increase	10
Mood elevation	13
Improved performance	14
Side effects on maintenance dose	2

In the 16 patients who were treated with Marsilid from the beginning (TABLE 2), 150 mg. per day was given with no other medication. Fourteen patients have shown a marked improvement that has been maintained throughout the 2 to 12 months of therapy. They felt better, ate more, gained weight, and felt greater freedom of movement and ability to undertake active exercise. Objectively, all have shown better performance of various movements, and several have now improved to the extent of being able to bend sufficiently to touch the floor.

The 2 patients who did not respond were in an acute phase. One other patient has developed an acute exacerbation under treatment. Marsilid did not help then, although previously the patient had been improved considerably by this drug.

In the early stages, minor side effects of the type seen with ganglion-blocking drugs were found. These were reduced by simultaneous administration of neostigmine. If treatment with Marsilid in smaller doses was continued, the side effects became less marked; only two patients showed side effects on maintenance therapy, and no serious toxicity was seen.

I believe that Marsilid is a very valuable form of adjuvant therapy in this type of disease. Its value undoubtedly would be greater if there were not the fear of jaundice. In view of this I have been undertaking some pilot studies with Marsilid analogues.

The chlorbenzoyl compound RO 4-1027 has been tried in doses between 150 and 300 mg./day (TABLE 3). At the lower dose level, less than 25 per cent of the patients showed a response less dramatic than that seen with Marsilid. Increase in the dose showed no further improvement, but produced marked side effects of sweating, muzziness, fatigue, and headaches, so that it became necessary to discontinue treatment. These patients subsequently showed a typical response to Marsilid, and are themselves quite convinced that the effect of Marsilid is better than that of RO 4-1027.

Two other compounds have been given for too short a time and to too few patients for full assessment. However, the results with RO 4-1340 (the alanine compound) appear promising, while small doses of RO 4-1038 (the serine compound) show an activity approaching that of Marsilid. Unfortunately, larger doses of this latter compound produce marked hypotension.

TABLE 3
RO 4-1027 IN ANKYLOSING SPONDYLITIS

	Treated	Improved	Stopped (No improvement side effects)
RO 4-1027	9	2	7
Iproniazid (same group)	7	6	1
Iproniazid (original series)	16	14	2

I fully support Scherbel's view that the mode of action of this group of compounds in the rheumatic disorders is still poorly understood. Michotte[2] has suggested that there is an excessive destruction of noradrenaline in this group of diseases, and it appeared possible at one time that Marsilid, acting as an amine oxidase inhibitor, was restoring this state to normal. However, in view of Axelrod's recent work, this explanation appears unlikely, at least in its simple form. I feel that the amine oxidase inhibitors act by producing mood elevation and pain tolerance rather than acting on the primary disease process, but that iproniazid is itself a valuable adjuvant in therapy of rheumatic disorders. We should, however, search for further compounds having similar activity with less toxicity and fewer side effects.

Acknowledgment

I express my gratitude to H. Coke for permission to quote from his results.[1]

References

1. COKE, H., S. E. FURBER & H. KERR-TAYLOR. 1959. Rheumatism. In press.
2. MICHOTTE, L. J. 1958. J. Belge. méd. phys. rheum. **13:** 183.

EXPERIENCE WITH JB-516 AND OTHER PSYCHOCHEMICALS

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Food, drink, and drugs can alter man's mood and behavior reactions to exogenous physical and endogenous biochemical environmental factors. Man has attempted to harness these forces by law, custom, shelter, clothing, and the application of knowledge derived from experience and experiment. This represents human effort to gain immunity from environmental stress. Certain obvious behavioral diseases such as general paralysis of the insane (G.P.I.), pellagra, and phenylpyruvic oligophrenia have been removed from the psychiatric area by specific drugs and nutritional modifications. Disturbances of less obvious organic structure are treated by incarceration, shock therapy, analysis, and other forms of psychiatric care. Other behavioral states may respond to sedative, hypnotic, tranquilizing, or stimulating drugs. These drugs are used and abused.[1]

The problem of anxiety has received much pharmacological attention; depressant, sedative, and tranquilizing drugs are legion. However, only the dextrorotary amphetamines and a few other relatively short-acting compounds are available to the depressed patient.

D-Amphetamine is a powerful and rapidly acting stimulant. However, sleeplessness, appetite suppression, other sympathomimetic effects, and rapid drug tolerance limit its usefulness in patients requiring euphoria and release from depressive reactions. It is effective only in mild cases of depression. D-Amphetamine is a relatively weak inhibitor of monamine oxidase.

The synthesis of potent, long-lasting monamine oxidase inhibitors has focused attention on them as highly effective experimental tools for the pharmacologist in the laboratory and in the clinic. Clinical trials with iproniazid have established its use in psychotherapy.[2] Laboratory screening of a large number of hydrazines[3] made available a new potent agent for these studies, namely, JB-516 (1-phenyl 2-propylhydrazine, Catron*) It is therefore important to translate this laboratory information into dynamic clinical usefulness. The following study was conducted with ambulatory geriatric patients in a chronic disease hospital and with patients treated in an office medical practice. Of the 79 patients treated with JB-516, the average duration of therapy was 6 months. A few patients were treated for as long as 1 year.

Experimental Design

To determine the clinical activity of JB-516, the following five procedures were followed:

(1) Based on toxicity data obtained in experimental animals, initial dose levels used for clinical trials were 12.5, 25, 50, and, in a few cases, 75 mg./day. From these preliminary dose-finding studies we selected 12.5 mg. daily as the dose level with the best therapeutic index. In the major portion of the study 12.5 mg./day was used.

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* Lakeside.

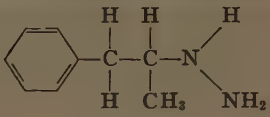
(2) The drug was then evaluated in a controlled placebo/drug study.

(3) Biochemical and clinical observations were followed closely to detect the presence or absence of toxic reactions.

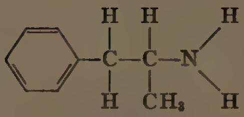
(4) JB-516 was evaluated in 79 patients. L-Ephedrine, D-amphetamine, and L-amphetamine, which are structurally related and within the same parameter of biological behavior, were used in a comparative study (TABLE 1).

TABLE 1

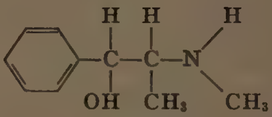
MOLECULAR STRUCTURES



JB-516



Amphetamine



Ephedrine

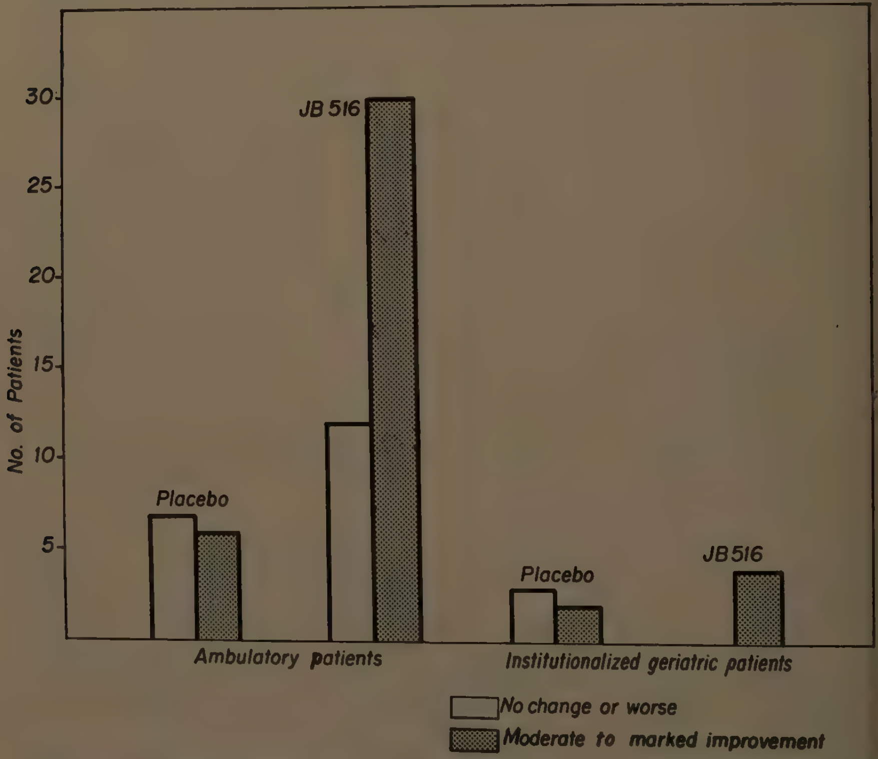


FIGURE 1. Results obtained with JB-516 in depressed patients (average dose of 12.5 mg./day).

(5) Eight normal subjects served as controls and were observed in a crossover study of JB-516 and the comparative compounds.

Results

Thirteen depressed ambulatory patients were treated with placebo and 42 were treated with JB-516. A significant number of the JB-516-treated patients responded with a satisfactory antidepressant effect. A similar effect was noted among institutionalized geriatric patients (FIGURE 1).

FIGURE 2 indicates a satisfactory response in postinfectious asthenia in ambulatory patients and asthenia in the geriatric institutionalized patient. Again, a significant response to JB-516 therapy can be seen.

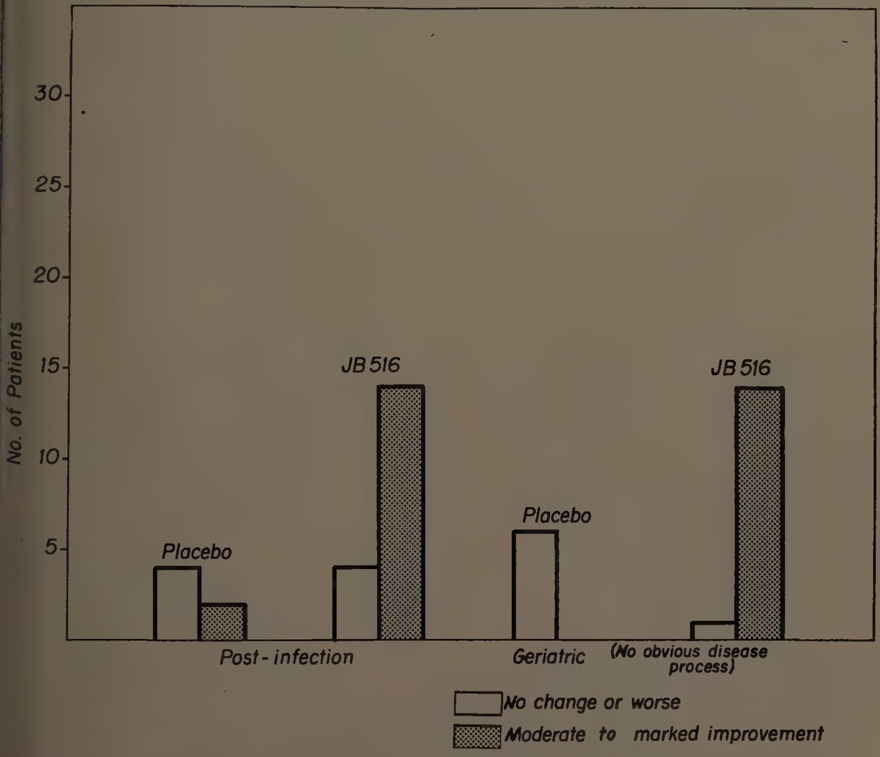


FIGURE 2. Results obtained with JB-516 in asthenia (average dose of 12.5 mg./day).

The comparative effects of JB-516 and structurally related drugs on mood elevation, increased activity, and appetite are indicated in TABLE 2. JB-516 differs from the comparative drugs in the lack of sympathomimetic side effects and the failure to influence appetite.

TABLE 3 illustrates the marked differences in time of onset of mood elevation between the 3 comparative drugs and JB-516. The side effects characteristic of JB-516 were seen only at higher doses.

TABLE 4 illustrates the central stimulating effect, the duration of activity after the final dose, and the problem of drug resistance of JB-516 and the comparative compounds in 8 normal subjects.

The delayed onset of effect and the prolonged duration of activity after the final dose are characteristic features of JB-516.

Discussion

A cautious determination established 12.5 mg./day as a safe and effective clinical dose. At this dose level there were no side effects in any of the patients. At a higher dosage (25 mg./day) there was some early-morning wakefulness in a few patients. In a few instances still higher doses were attempted (50 or 75 mg.), and at these levels there occurred some muscle weakness, unrelated to hypotension, and occasional tremor and muscle twitching.

TABLE 2
COMPARATIVE CLINICAL RESPONSES

Drug	Total daily dosage (mg.)	Mood elevation	Increased activity	Appetite suppression	Sympathomimetic side effects (tachycardia tremors, nervousness)
D-Amphetamine sulfate	5	++++	++++	++++	++++
L-Amphetamine sulfate	10	+	++	++++	++++
L-Ephedrine sulfate	50	++	++	++	++++
JB-516	6.5 to 12.5	++	+++	+	+

TABLE 3
MOOD ELEVATION IN DEPRESSED PATIENTS

Drug	Total daily dose (mg.)	No. of patients	Onset of effect	Result	Side effects
D-Amphetamine sulfate	5 to 20	32	Rapid	++++	Nervousness, tremor tachycardia
L-Amphetamine sulfate	10 to 20	23	Rapid	++	Minimal
L-Ephedrine sulfate	50	44	Rapid	++	Minimal
JB-516	12.5 to 50	79	4 to 14 days	++	At high doses there was early awakening, muscular twitching, muscular weakness.

TABLE 4
COMPARATIVE FINDINGS IN 8 NORMAL SUBJECTS

Drug	Total daily dose (mg.)	Onset	Central stimulation	Duration of activity after final dose	Drug resistance
D-Amphetamine sulfate	5	Rapid	++++	4 to 24 hours	+++
L-Amphetamine sulfate	10	Rapid	++	4 to 24 hours	++
L-Ephedrine sulfate	50	Rapid	++	3 to 6 hours	++++
JB-516	12.5	4 to 14 days	++	3 to 10 days	+−

There is a wide and puzzling gap between the powerful pharmacologica effect on animals and the lack of expected rapid results in clinical trial. If conclusions could be drawn from experimental animal studies, JB-516 should cause abrupt and marked effects in human subjects. Instead, the effect at our test dose level was a slowly achieved and smoothly maintained elevation of mood. JB-516 does not cause the erratic behavior, the pitch of immediate abnormal hyperactivity, and the subsequent undesirable depression characteristic of amphetamine and ephedrine. No respiratory manifestations or evidences of sympathotonia were seen even at high dose levels. A remarkable muscular weakness was noted in 2 subjects treated for 10 to 14 days with 75 mg./day. The weakness was most marked in the legs, and there was no evidence of hypotension even with change of position. The weakness disappeared within 48 hours following discontinuance of therapy.

The disparity between results obtained in the pharmacology laboratory and those in the clinic may be a reflection of a comparison of high pharmacological doses versus safe, conservative, clinical doses. This may mean that clinical doses cannot achieve the sudden high degree of monoamine oxidase inhibition necessary for the dramatic effects achieved in the laboratory. In addition, it is likely that other metabolic pathways for amine inactivation are present.[4] Other mechansims may also account for the stimulant effect of JB-516.

Since the pharmacodynamic activity of this class of drugs is not vivid, and since it is compromised by psychic forces and must be determined by subjective responses, an evaluation becomes difficult. However, this study indicates the clear clinical pharmacodynamic activity of JB-516. Its usefulness would appear to be greatest as an antidepressive agent in those patients who require such help until an overwhelming realistic situation is resolved or analytic procedures can provide the emotional recognition of the problem.

One cannot emphasize too strongly the importance of correct individual dosage. It is obvious that patients vary in their external behavioral manifestations as well as their responsiveness to drugs and their dosage forms. We see depressed patients unaffected by 30 to 50 mg. of D-amphetamine sulfate per day; on the other hand, excitable individuals spend foodless days and sleepless nights on 5 mg. of the same drug. There are many varied types and constitutions of human beings, and their responses to a dose of a given drug will vary in as many different ways. When a drug has been evaluated with the most exacting techniques by well-qualified observers, only relative standards will have been established. The practitioner is then required to titrate the drug to each individual patient within the established therapeutic *versus* toxic limits. The therapeutic index of the drug[5, 6] serves to limit or broaden its usefulness. It establishes a general safety area within which the dose for each individual patient may be found. Often, knowledge of the patient may help, but sometimes trial and error is required. It is important that the drug be not discarded because of overeffect or apparent lack of effectiveness before the optimum dose has been found. The clinical pharmacologist establishes the dynamic activity of the drug, the therapeutic index, and the safe dose range; the practicing physician establishes the specific dose for each individual patient.

At higher dosage, JB-516 may cause side effects that are not present with a daily dose of 12.5 mg. At this safe dose level, JB-516 is a good antidepressive agent for patients requiring sustained activity during periods of stress until the causative difficulty is solved or fantasy problems are properly evaluated. In addition, there appears to be an important role for this drug in postinfectious asthenia, in ambulatory patients, and in the asthenia of undetermined etiology observed in institutionalized geriatric patients. A sensible, sane, orderly, and controlled behavior is achieved in those patients responsive to the established safe and effective dose of JB-516.

Conclusions

JB-516 has been clinically screened at 3 dose levels for side effects, activity, and dosage.

The safe dose has been established as 12.5 mg./day.

Comparative studies with related compounds D-amphetamine, L-amphetamine, and L-ephedrine indicate significant clinical differences between JB-516 and the reference compounds.

A clinical response was achieved in a significant number of patients at the determined safe dose range.

No evidence of toxicity was found during a series of biochemical studies.

References

1. Pomeranze, J. 1957. Realism in the use of tranquilizing drugs. J. Am. Geriatrics Soc. **5:** 823.
2. Orland, I. & N. L. Comar. 1958. The adjunctive use of iproniazid in psychotherapy. Diseases of Nervous System. **19:** 182.
3. Biel, J. H., A. E. Drukker, P. A. Shore, S. Spector & B. B. Brodie. 1958. Effect of 1-phenyl 2-hydrazinopropane, a potent monoamine oxidase (MAO) inhibitor on brain levels of norepinephrine and serotinin. J. Am. Chem. Soc. **80:** 1519.
4. LaBrosse, E. H., J. Axelrod & S. S. Kety. 1958. O-methylation, the principal root of metabolism of epinephrine in man. Science. **128:** 593.
5. Pomeranze, J. & R. J. Gadek. 1957. Clinical appraisal of drugs. New Engl. J. Med. **257:** 73.
6. Pomeranze, J. & R. J. Gadek. 1957. The therapeutic index of JB 323 in peptic ulcers. Am. Practitioners and Dig. Treatment. **8:** 73.

Discussion of the Paper

V. John Kinross-Wright (*Baylor University College of Medicine, Houston, Texas, and Houston State Psychiatric Institute, Houston, Texas*): Pomeranze is to be congratulated upon his careful and conservative appraisal of JB-516. In his paper he makes the following points, which I believe deserve re-emphasis. First, monoamine oxidase inhibitors such as JB-516 are quite different in their clinical effects from the amphetamines, despite opposing findings in animal pharmacological work. However, it might be of interest to note that we have obtained marked amphetaminelike effects within 1 hour that persisted for as long as 36 hours with another monoamine oxidase inhibitor, SKF-385. This drug was given in large doses (1.5 mg./kg.) to 3 normal subjects in a special study. As Pomeranze has suggested, the size of the dose is probably the critical factor in this case.

Second, Pomeranze has stressed the marked individual variation of patients in their response to these drugs. Finally, he has indicated that side effects, sometimes of an unusual nature, may be encountered.

Our experience has been restricted to 29 patients who were moderately to severely depressed. In most of these, electroshock treatment had either been tried unsuccessfully or had been contemplated.

In the series there were 13 cases of endogenous depression, of whom 9 achieved substantial to complete relief of their symptoms with JB-516. There were 10 cases of reactive depression, of whom 5 showed substantial or complete relief of symptoms. The remaining 6 cases had depressions associated with schizophrenia or organic brain disorder. Two of them were adequately relieved.

Those patients who responded showed in general less anxiety and agitation than the failures; that is, they tended to conform to the simple retarded type. Otherwise, there were no clues to explain the inconsistency of the results.

One patient who had a previous history of manic disturbance many years before passed rapidly from depression to mania on the thirty-fourth day of treatment with JB-516. The condition did not respond to withdrawal of the drug.

A dosage of 12.5 mg. twice daily was usual. In a few patients as much as 25 mg. twice daily was tried, with no apparent advantage. Once a therapeutic response had been gained, dosage was reduced to 12.5 or 6.25 mg. daily, in most cases with maintenance of benefit. The duration of treatment required appears to be an individual matter. Improvement occurred characteristically between the tenth and fourteenth days of treatment. In one case it happened on the fourth day, and in another it was delayed until the fourth week. In the latter case it can probably be assumed that factors other than the drug were paramount.

Side effects were rarely seen during the first 2 weeks of treatment. They appeared to develop concurrently with a therapeutic response indicating, presumably, that a considerable level of monoamine oxidase inhibition throughout the body had been achieved. Six patients experienced some fall in blood pressure during treatment; only in 2 did the fall exceed 30 mm. Hg (systolic). No patient complained of anorexia; indeed, most reported improvement in appetite. Insomnia was noted by some of the patients who made a good response to treatment, although this was preferred to the depressive symptoms. It was possible in all cases to achieve satisfactory nocturnal sleep by reducing dosage without a return of depressive symptoms.

One patient exhibited mild amphetaminelike symptoms of restlessness, tension, anorexia, and dryness of mouth on the first day of treatment (25 mg.). It was not possible to determine whether this was a placebo effect or the kind of behavior seen in animal experiments.

Liver function tests and blood cytology were uninfluenced by JB-516 in the 8 of these patients who were tested at weekly intervals.

Three patients developed reactions which are worth reporting in greater detail:

A 47-year-old white woman with endogenous depression received JB-516 (25 mg./day) for 15 days. By the end of that time she developed weakness and numbness of the extremities, dizziness, and ataxia, and complaints of blurred central vision, with green scotomata. Blood pressure dropped from 190/80 to 136/72. Visual and neurological examination was negative except for muscular weakness of the lower extremities. JB-516 was withdrawn, and two days afterward the patient was symptom-free but still depressed.

The second case, a 28-year-old white female, was diagnosed as reactive depression and had responded only slightly to a course of electroshock treatment. A daily dose of 12.5 mg. JB-516 produced an excellent response. However, in the fifth week of treatment she developed ataxia, slurring of speech, and nystagmus, most marked on lateral deviation of the eyes. Neurological findings were again negative except for weakness and a positive Romberg test. The drug was withdrawn, and the disturbance gradually receded over a period of 6 weeks.

A 54-year-old white man received varying dosages up to 25 mg. twice daily for treatment of an endogenous depression. He made good progress until the twenty-sixth day, when he developed a maculopapular rash of the trunk and arms, blurring of vision, dryness of mouth, and marked dizziness. He exibited gross orthostatic hypotension. Symptoms cleared when the dose was halved, but returned a few days later. It was necessary to reduce the drug to 12.5 mg./day before complete abolition of the hypotension could be achieved.

The following conclusions may be drawn from the experiences of Pomeranze and myself:

JB-516 is an effective antidepressant that appears to be more effective with the more typical depressions. The optimal dose is approximately 12.5 mg./day. While sympatholytic effects are marked in some cases, these are generally less than with iproniazid. There is no evidence of hepatic toxicity. Inhibition of monoamine oxidase is capable of evoking widespread changes in biological functions, and considerable clinical caution is necessary to exclude all but the desirable ones.

THE USE OF JB-516 IN NEUROMUSCULAR CONDITIONS IN PEDIATRICS*

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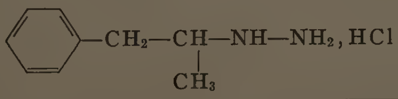
Introduction

The clinical evaluation of a drug is difficult because of the multiplicity of factors responsible for clinical improvement or regression. In many neurological conditions, for example, epilepsy or multiple sclerosis, spontaneous remissions and exacerbations commonly occur. At times a drug may appear to benefit a given condition; at other times it may fail to do so in the same patient. This does not necessarily mean that the drug is without benefit, but it might mean that its beneficial effects were manifest only under certain circumstances, as in conjunction with another drug. The same drug, or combination of drugs, in the presence of unfavorable concomitant factors, for example, during the tension of a menstrual period or during the stress of an upper respiratory infection, might have its beneficial effects blocked. Frequently, a drug that is thought to be beneficial is discontinued without an exacerbation of the symptoms. In this event one may have difficulty deciding whether the original improvement was fortuitous and unrelated to the drug in question, or whether the drug was not only beneficial, but that its benefits were irreversible or permanent. Double-blind studies do not always rule out drug efficacy. The fact that a placebo may be equally successful does not rule out the possibility that the drug under question may also have a beneficial pharmacological effect.

In clinical medicine the giving of a pill has therapeutic implications that transcend the mere pharmacological effects of the drug. Many psychological and social factors must also be considered as components of the therapy. Not only is the patient susceptible to suggestion and emotional influences, but the observing physician is also subject to the same influences. How else can one explain the numerous erroneous reports in the literature of almost miraculous effects of many drugs, reports made by reputable, honest observers? The use of double-blind studies has been helpful in eliminating some of the pitfalls to which human observation is prey, but there is no substitute for critical objectivity, skepticism, and clinical experience in evaluating drug efficacy.

Pharmacology of JB-516

JB-516 (Catron†), a cogener of amphetamine, is chemically (1-methyl-2-phenyl)-ethylhydrazine hydrochloride and has the following structural formula:



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* The work reported in this paper was supported in part by grants from the United Cerebral Palsy and Muscular Dystrophy Association, Inc. Chicago, Ill., and Lakeside Laboratories, Inc., Milwaukee, Wis.

† Lakeside.

Pharmacologically, it inactivates monoamine oxidase (MAO). The latter compound plays a part in the inactivation of serotonin, which is important in brain physiology. The administration of JB-516 is followed by a rise in serotonin and norepinephrine levels in the brains of rabbits. It has been postulated that inhibitors of serotonin inhibitors may act clinically as central stimulants.

JB-516 also has a protective action against electroshock convulsions in rats. Like the amphetamine derivatives, it reduces the need for sleep; however, unlike the amphetamines, it does not depress the appetite; in fact, it may improve it. The drug also acts much longer than the amphetamines and may, in fact, be effective for from 24 to 96 hours. Not only is its central effect prolonged, but it is not followed by a secondary stage of depression after stoppage. It also tends to lower blood pressure. In humans it has been reported to be of value in depressive states. It has also been reported to be a potentiator of other drugs, particularly those of the promazine type, and to be valuable in reducing hypertension.

TABLE 1
JB-516 IN NEUROPEDIATRICS
(110 cases)

Diagnosis	Benefit*				
	No.	E	M	O	A
Cerebral palsy	16	0	0	16	0
Epilepsy	34	0	3	28	3
Emotional	60	9	14	36	1

* E, excellent; M, moderate; O, none; and A, aggravated.

Experimental Population and Methods

The experimental population involved in this study consists of 110 patients with a variety of neuromuscular diseases (TABLE 1). Sixteen patients had various forms of cerebral palsy, 34 had epilepsy of various types, and the remaining 60 had emotional and behavioral disturbances of different degrees and types. The patients were in part from the Children's Neurology Service of Cook County Hospital, Chicago, Ill., and from private practice. These cases, varied in age from 3 months to 20 years, had for the most part been under observation for prolonged periods, and had been on various drug regimes to which they had been generally refractory.

For purposes of this study, the behavioral disturbances were divided into three general classes:

(1) *Purely functional or symptomatic.* These were otherwise normal children in whom the emotional and behavioral problems may have been due to such things as sibling rivalry or broken homes, and consisted of hyperactive behavior, tantrums, infantile regressions such as thumbsucking, enuresis and, often, autistic withdrawal. Routine neurological examination revealed no evidence of organic brain disease. In most of these children the electroencephalograms (EEGs) were either normal or showed 14- and 6-per-sec. positive spikes.

(2) *Functional disturbances in patients with organic brain disease.* In this

group were included patients with obvious organic brain involvement such as hemiplegia, mental deficiency, and/or epileptic symptoms. In many of these children, functional behavioral disturbances were engrafted because of familial rejection, frustrating experiences due to physical disabilities, or other social and emotional causes. In this group of patients the behavioral disturbances were similar to those in the first group, hyperactivity and aggressive or autistic behavior predominating. Behavioral episodes such as tantrums were generally triggered in this group of patients by punishment or frustrations and, in this regard, were different from the behavior of the patients in the next group.

(3) *Organically driven behavior in patients with organic brain disease* (*OBD*). Children in this group were the most severely involved, and their behavior was also characterized by marked hyperactivity. However, this hyperactivity had the characteristics of "organic drive," with episodic rages and tantrums, apparently untriggered by exogenous stimuli. In these patients, the behavioral episodes often resembled epileptic equivalents or psychomotor spells, and a patient was often unaware of having experienced such an episode. Between periods of rage, such a patient's behavior often approached normal. The electroencephalograms of this group of patients were generally very abnormal and frequently had focal temporal spikes. Behavioral disturbances were common following encephalitis.

In addition, behavioral disturbances were classified according to three grades of severity: mild, moderate, and severe. The mild cases were those in whom there were hyperactivity, tantrums, and asocial behavior not sufficiently severe to cause a child to be dismissed from school or to be ostracized by his playmates. The moderate cases were those in which behavior had reached a point where social difficulties were being encountered. In this group were children whose behavior was characterized by withdrawal from reality or by aggressive tendencies, and who could no longer attend school because of these difficulties. The emotional problems of these children often interfered, to some degree, with their intellectual functioning. The behavior problem, although severe, was not considered irreversible. The severe group included those children in whom behavioral problems had reached a point of near or clear irreversibility; it included the severely autistic children totally out of contact with their environment, or the prepsychotic and psychotic. Most of these children were those eligible for institutional care.

Method

Since the present study was in the nature of a screening experiment, the double-blind technique was not employed. At the beginning of the experiment the drug was given to the parent with a code number label and without much comment as to what to expect. When it became apparent that sleeplessness or hyperirritability might occur within a week after starting treatment, the parents were told that in such a case the drug was to be given every other day instead of daily. This was done in order to prevent prejudice against further use of the drug on the part of the parents or patients. If the drug seemed to be of benefit, it was stopped and then restarted; often it was replaced by a placebo in order to determine whether the pharmacological effect was real or imagined.

The estimation of improvement in a clinical experiment cannot, unfortunately, always be quantitated. One must therefore often use subjective estimates of improvement when objective ones are not available. In patients with seizures, the frequency and severity of the seizures was considered to be a fairly reliable index of improvement. A reduction in spells of 75 per cent or more was considered excellent, 50 to 75 per cent moderate, and less than 50 per cent of no benefit. In patients with cerebral palsy the measure of improvement was objectively quantitated by measuring performance: for example, by counting the number of steps taken before and after the drug, or the number of pegs accurately replaced in a given amount of time.

In the area of emotional and behavioral problems much greater subjectivity was employed in measuring improvement. The criterion for benefit in this group was an improvement that allowed the child to be removed from one grade in the aforementioned classification to the one above it. If this occurred it was considered as excellent benefit. If the improvement was not this great but still was evident, it was considered as being moderate improvement. If the improvement was slight, questionable or absent, it was considered as no improvement. Evaluation of improvement was based upon the reports of the parents, teachers and therapists and from personal observation of the examiner. Admittedly, all of these methods of scoring are open to objection, but in a clinical study of this sort they are the only practical methods available. In the course of the follow-up of these patients, electroencephalograms, psychological evaluations, and social histories were taken, and routine blood, urine, and other specific tests were done as indicated. We recognize that the improvement in many of our patients might have been concomitant with improved attitudes in the parents and with the psychiatric improvement in the patient that often occurs when new therapy is attempted. In a better-controlled study one would probably find improvement less frequently or to a lesser degree than this study might indicate.

Dosage

The drug was supplied in liquid form containing 12½ mg. per teaspoon and in scored tablets containing 6.25 mg. and 3 mg. per tablet. Although in some instances the drug was given twice daily, in most instances it was given once a day. If any toxic side effects appeared the drug was either reduced in dosage or the interval between doses was increased to 2, 3, or even as many as 7 days. In most instances a single dose was given every morning and, in many instances, 3 times weekly.

Results

Cerebral palsy. As may be seen from TABLE 1, the drug was of no benefit in reducing muscle tension, spasticity, or rigidity in the 16 patients with various types of cerebral palsy. The various subdivisions and diagnoses are not broken down for this group, since the drug was of no benefit in any of them. The addition of JB-516 caused drowsiness in several patients who were receiving sedatives such as the barbiturates, but this was considered to be due to the potentiating effect of the drug on barbiturates rather than due to its innate pharmacological effect. When given alone, the drug did not have this soporific effect. One must therefore conclude that the drug is of no value as a relaxant drug in cerebral palsy.

Epilepsy. In the group of 34 epileptics were patients with *grand mal, petit mal,* and with vegetative, focal, and other types of spells. There were 6 patients with the idiopathic form of epilepsy and 26 with organic brain disease. The drug was of moderate benefit to but 3 of this group of 32 patients. These were all patients with organic brain disease who were receiving other anticonvulsant drugs. One of these was a patient with akinetic spells, and the other two had focal and vegetative spells. The addition of JB-516 reduced the number of these spells by about 50 per cent. In no patient to whom the drug was given alone, however, was there any noticeable anticonvulsant effect. In 3 patients there was an aggravation of the *grand mal* seizures. These also were patients with organic brain disease who were receiving other anticonvulsant drugs. It appears, therefore, that JB-516 is of limited value as an anticonvulsant and that it might, in fact, tend to precipitate *grand mal* seizures. Any antiepileptic value that it may have might be due to its possible potentiating effect on concomitantly given anticonvulsant drugs.

TABLE 2

JB-516 IN EMOTIONAL AND BEHAVIORAL DISTURBANCES

	Benefit*				
	No.	E	M	O	A
Total	60	9	14	8	1
Mild	18	5	4	36	1
Moderate	28	3	8	17	0
Severe	14	1	2	11	0

* E, excellent; M, moderate; O, none; and A, aggravated.

Emotion and behavior. TABLE 2 shows that the area of greatest benefit of JB-516 is in the group with behavioral and emotional disturbances, primarily those of mild to moderate degree. Of the total of 60 patients in this group, 9 evinced an excellent response and 14 a moderately good one for a total of 23 (38 per cent) showing improvement; in only 1 was there an aggravation of symptoms. When these disturbances are divided according to severity (TABLE 2) it is seen that of 18 patients with mild involvement there were 9 who showed improvement (50 per cent). Of 28 with moderate involvement, there were 11 (40 per cent) who improved, whereas only 3 of 14 (21 per cent) severely involved patients were benefited.

TABLE 3 shows that JB-516 is of greatest value in patients with functional behavioral disturbances with or without the presence of organic brain disease. It was of benefit in 5 of 11 children without organic brain disease and in 14 of 28 children with organic brain disease, or a total of 19 of 39 (48 per cent) of the patients with functional behavioral problems. In the group of 21 patients in whom the behavioral disturbances was considered to be entirely endogenous and due to organic brain disease, there was improvement in but 4 (19 per cent). In only 1 patient was there an aggravation of symptoms, and this was in a postencephalitic with "organic drive."

Improvement, when it occurred, was usually delayed from 2 to 5 days and was not as rapid as that which follows the successful use of dextroamphetamine sulfate (Dexedrine*). Improvement in behavior generally consisted of a diminution of irritability, hyperactivity, and irascibility. The patients generally had a longer attention span and seemed to be more in contact with their environment and responded better to it. In many of the children in this group who were helped there had been a paradoxical reaction to barbiturates that tended to increase the excitement instead of decreasing it.

As indicated above, most behavioral disturbances of children are characterized by hyperactivity and hyperirritability. Depression as an outstanding symptom in behavioral disturbances of children is uncommon except for autistic reactions. In this group of patients there were, however, 4 with depression, 3 of whom were benefited.

In assaying a central stimulant drug it might be well to compare it with Dexedrine, which has a similar action on behavior and with which a greater experience has been available. JB-516 seems to have an order of effectiveness close to that of Dexedrine, although its effectiveness, as indicated above, is not as rapid or dramatic. Unlike Dexedrine, it did not affect the appetite or cause the same degree of exacerbation of irritability. The drug seemed to have a cumulative effect and was apparently retained in the body for at least 24 hours. This became evident from the fact that the side effects, and often the benefits, would not become apparent for from 2 to 7 days. In some instances, therefore, it was found necessary to lengthen the period between dosages from 2 to 7 days in order to retain the benefit of the drug without experiencing its side effects. In order to illustrate some of this beneficial effect, 3 illustrative case reports follow:

TABLE 3

JB-516 IN EMOTIONAL AND BEHAVIORAL DISTURBANCES

	Benefit*				
	No.	E	M	O	A
Total	60	9	14	36	1
Function without OBD	11	2	3	6	0
Function with OBD	28	6	8	14	0
OBD	21	1	3	16	1

* E, excellent; M, moderate; O, none; and A, aggravated.

Case No. 1. K.F., 4-year-old white male with congenital mild left spastic hemiplegia due to birth residue and with blindness due to retrolental fibroplasia and functional behavioral disturbance. Improved on JB-516; improvement continuing even after stopping drug.

Behavioral problems in this blind, hemiplegic boy had been present for 3 years and consisted of temper tantrums, bed rocking, head banging, hand gazing, head holding, toe walking, infantile personality, and autistic behavior. The boy was markedly overprotected and frustrated because of parental confusions and attitudes; his I.Q. was 55 per cent, and his EEG very abnormal, with spike seizure and slow wave foci in the left occipital area. Clinical impression: autistic behavior of moderate degree superimposed on child with organic brain disease, hemiplegia, and blindness due to retrolental fibroplasia.

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* Smith, Kline & French.

Dexedrine (2½ mg. twice daily) caused him to become very nervous and caused anorexia, which necessitated withdrawal. Because of abnormal EEG, he was given diphenylhydantoin (Dilantin*) but without benefit. Chlorpromazine (Thorazine†) and other ataractics were of no benefit. At the age of 6 years, after 2 years of observation, there was not much change in general attitude. At this time the patient was started on JB-516 (12½ mg./day). Within 1 week there was marked improvement; the child was less autistic; he began to respond to people about him; hyperactivity and tantrums decreased. About 2 weeks after start of drug, he began to get up earlier than usual in the morning and to stay up later at night; therefore, the dose was cut to 12½ mg. every second day with cessation of sleeplessness. After 3 months he was able to cooperate sufficiently to be able to enter into a physical therapy program and into a nursery school that had refused to accept him previously. About 4 months after beginning of JB-516 therapy the patient's mother noticed the same sleeplessness beginning, and the drug was withdrawn entirely as a trial. There was no loss of benefit, and the child continued to improve. It was felt that the change in parental attitudes was responsible for the continuing improvement. The child has continued in nursery school and on the therapy program. The estimate of improvement was excellent.

Case No. 2. G.R., a 10-year-old white male with moderate functional behavior disturbances and school difficulties. Improvement on JB-516.

The patient had been perfectly well until the age of 8 years, when he sustained a fracture of the right humerus, with nerve complications and elbow contractures and, as a result, a forced change in handedness. School difficulties had begun 1½ years before, with hyperactivity, aggressive behavior, short attention span, and tendency to become accident-prone. There was sibling rivalry with a brother who was 1 year older. The patient gradually became worse, developed pica, eneuresis, and soiling, and finally became somewhat autistic and his withdrawal from school became necessary. His I.Q. at this time was 75 per cent, but this was considered to be below his true potential. His EEG was normal. Diagnosis was moderately severe functional behavior disturbance with aggressiveness, hyperactivity, and infantile regression. JB-516 treatment was started (6½ mg. morning and evening); after 10 days on the drug his behavior began to improve, and eneuresis and soiling stopped. At this time he began to feel sleepy. The drug was withdrawn and his sleepiness disappeared, but he began to wet the bed again. The drug was restarted, but the dose was cut to 6½ mg. every other day; there was no recurrence of sleepiness, but behavior again improved after 10 days, with stoppage of eneuresis and soiling. After 2 months on the drug his gradual improvement was such that he was readmitted to classroom on a trial basis. The estimate of his improvement was also excellent.

Case No. 3. J.S., a 14-year-old white female with progressive organic brain

* Parke, Davis.
† Smith, Kline & French.

disease with akinetic spells, hyperactive behavior, and mental deficiency. Moderately good improvement in seizures and behavior on JB-516, with apparent remission in progress.

This girl had apparently been normal until the age of 5 years, when akinetic seizures began. In spite of various types of medication she grew progressively worse; she began to deteriorate mentally and developed behavior problems. She exhibited hyperactivity, short attention span, aggressiveness, tantrums, and refusal to obey orders. When seen at the age of 14 years she had a very abnormal EEG with multiple seizure foci, principally in the right occipital area. She had previously been on phenobarbital, which had caused a paradoxical reaction, and on methsuximid (Celontin*), trimethadione (Tridione†), 3-ethyl-5-phenylhydantoin (Peganone†), 5-phenyl-5-ethyl-hexahydropyrimidine-4:6 dione (Mysoline‡), and Dilantin, without any effect on her behavior or spells. On Dilantin (3 gr./day) she was still suffering an average of 6 akinetic spells per day. Diagnosis was progressive brain disease, possibly postencephalitic, with behavior disturbances of severe degree due to organic drive. At this time JB-516 (12½ mg. morning and evening) was added to the Dilantin. At the end of the third day there was reduction in the frequency of seizures to about 2 per day. The behavior improved greatly, with a decrease in hyperactivity, aggressiveness, and destructiveness. The patient became more obedient and was generally happier. By the tenth day she acted much more alert than previously, but had begun to get up at 5 A.M., and to go to bed after midnight. JB-516 was therefore cut to 1 dose of 12½ mg. every morning, with a subsequent amelioration of her sleeplessness. After 2 months on the drug the patient was still improving in behavior, and spells were now occurring about twice a week. After 4 months the drug was stopped experimentally. Within 1 week the spells had increased to about 6 per day, and behavior began to deteriorate. The drug was therefore restarted at 12½ mg. once daily. After 11 months on the drug, the seizures are occurring about 3 times per week, and the behavior remains moderately improved. Intelligence has not changed; although the patient is still not ready for school, there has not only been a stoppage of the progression of the disease, but some evidence of improvement. The estimate of improvement is moderately good in seizures and in behavior.

Side Effects

The most common side effect was sleeplessness. This would evince itself by the tendency of the patient to arise earlier in the morning, to eliminate nap periods, and to go to bed later at night. Frequently it would require 2 to 7 days for the appearance of such a side effect. From TABLE 4 it may be seen that this side effect occurred 27 times in 110 cases. This is a rather high incidence for a side effect, and, it can be partially explained by the fact that it was our policy to start with low doses and to increase them in doubtful cases until this side effect appeared. This symptom could be eliminated easily either by reducing the dosage or, even better, by lengthening the period between dosages so that the drug would be given every 2, 3, 4, or 5 days; in 2 instances it was necessary to give the drug but once weekly. In 7 patients this sleeplessness was associated with hyperirritability and aggravation of the aggressive symptoms. In 2 instances there was nausea and vomiting and, in one, there was ataxia. We were also surprised to see drowsiness occur in some patients (there were 7 of these) particularly those who were on other sedatives, which indicated that the drug might have had a potentiating effect. In one instance, however, drowsiness occurred when the drug was given alone. In no instance was there a skin rash, leukopenia or other blood dyscrasias, or urinary disturbances. Liver profiles done on 20 patients were normal. Hypotension was looked for but not noted.

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* Parke-Davis.
† Abbott.
‡ Ayerst.

Comment

JB-516 appears to be a useful drug in neuropediatrics. It is of no value as a muscle relaxant in cerebral palsy and is probably of little benefit in epilepsy; in fact, there is a possibility that it might aggravate *grand mal* spells. However, in the field of emotional and behavioral disturbances in children, it is of definite benefit. It is of least benefit in those patients in whom the behavior is characterized by "organic drive" with episodic, untriggered rages that simulate epileptic seizures. It is of greatest benefit, however, in behavioral disturbances of a functional nature with or without the presence of organic brain disease. In this category it was of benefit in approximately 50 per cent of the patients. It is more effective in mild and moderately involved patients and least effective in those with severe autism or psychoses. The drug compares well in efficacy with pipradrol hydrochloride (Meretran*) and iproniazid (Marsilid†). It resembles the amphetamine group in its action in that it appears to have a sedating effect in children with hyperactivity and short attention spans. It also seems to be effective in children in whom a paradoxical reaction to phenobarbital occurs. Unlike the amphetamines, it does not depress appetite nor cause severe insomnia and exacerbations of hyperactivity. Its rate of action was slower and its time of effective action much longer than that of the amphetamines.

TABLE 4
SIDE EFFECTS OF JB-516
(110 cases)

Sleepless	27
Sleepy	7
Increased activity	7
Nausea	2
Ataxia	1

No blood, urine, liver function or blood pressure changes.

Depressions as part of an emotional disturbance are less common in children than in adults. In 4 of the children in this series, depression was an outstanding symptom, and in 3 of these JB-516 was of benefit. Because of the relative safety of the drug it should be considered a valuable adjuvant to the treatment of functional emotional behavioral disturbances in children.

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* Merrell.

† Hoffmann-La Roche.

Summary

JB-516, an MAO inhibitor, is a relatively safe drug in the management of functional behavioral disturbances in children with or without organic brain disease. It improves the behavior of approximately 50 per cent of such patients. It is less valuable in behavioral disturbances characterized primarily by organicity and in those with psychoses. It is of no value as a muscle relaxant in cerebral palsy and of doubtful value as an antiepileptic.

Acknowledgment

I appreciate the supplies of JB-516 made available by Harvey Daiell of the Lakeside Laboratories, Milwaukee, Wis.

Discussion of the Paper

MICHEL D. STEIN (*Cook County Hospital, Chicago, Ill.*): It is axiomatic in medicine that one must beware of doing harm to one's patients by overzealous treatment. Although this axiom is always borne in mind in the evaluation of a new drug, it is not this possible harmful effect that is often a limiting factor; rather, it is the presence of minor but very undesirable side effects that may preclude its use.

If a drug is to be useful in clinical practice, its useful properties must far outweigh any unexpected and unpleasant side effects. This is particularly true in pediatrics, when such apparently trivial matters as an unpleasant taste, which cannot be disguised, may make impossible the use of a very excellent compound. Taste is not, incidentally, a problem with JB-416. Particularly when one is dealing with mood-ameliorating drugs, one must be especially careful of introducing new and unpleasant results or sensations.

In our experience with JB-516, it was noted that there was a small, but significant number of patients (approximately 33 per cent) who developed undesirable side effects with this drug. These reactions were, for the most part, of a relatively minor nature and were easily corrected by the proper adjustment of dosage. However, there were a very few patients whose side effects were of such a serious nature as to preclude use of this drug.

The most frequent side effect noted was sleeplessness. This was usually not insomnia or inability to sleep, but a desire for less sleep, which appeared as an earlier hour of awakening, a later hour of retiring, or the discontinuance of an afternoon nap. In the child with organic brain damage who is hyperkinetic this side effect resembles the paradoxical reaction to barbiturates that many of these children show, namely, sleeplessness rather than sedation. This could be easily corrected by reducing dosage to every 2 or 3 days or, if necessary, to as little as once every 5 to 7 days. Sleeplessness alone is not sufficiently troublesome to cause discontinuance of the drug. Paradoxically, a few children reacted by becoming excessively sleepy. This is also quite undesirable but, likewise, it can be corrected by adjusting the dosage schedule.

Nausea and vomiting, the only gastrointestinal symptoms noted, recurred in only 2 patients. Although these were relatively minor side effects, they could preclude the use of the drug.

Hypotension was not noted in any of our cases.

Among the possible serious side effects of this drug, the only ones noted in our patients were ataxia and increasing restlessness or hyperactivity. No hepatic involvement was noted, nor did any abnormalities of the blood develop during the study period.

Ataxia was noted in only one case, but this condition necessitates a temporary or even permanent withdrawal from the drug. An ataxic, or "drunk," child can be a menace to himself due to his inability to maintain balance. This is particularly true if the child is on other medications; because of a convulsive diathesis one must be wary of introducing a new source of balance and gait disturbance. However, it is to be emphasized that ataxia occurred only in this single case.

The only other serious side effect noted in our series was an increase in restlessness and hyperactivity in some of the children with organic brain damage. As this makes a previously bad situation even more intolerable, it constitutes an indication to withdraw the drug.

One other possible serious side effect noted was the possible aggravation of *grand mal* epilepsy in 2 patients whose seizures had previously been well controlled.

Two other points should be emphasized. First, in the pediatric population, depression is not as common an occurrence as in the adult population. However, in the depressed children treated with JB-516 the response was equally as satisfactory as has been reported today in adults. Second, there is no foolproof method of predicting which children will respond satisfactorily to JB-516; rather, this compound must be used on a trial-and-error basis.

In summary, the side effects of JB-516, although noted in approximately 33 per cent of the patients, are usually relatively minor in nature. Serious side effects noted were ataxia, increasing restlessness, and hyperirritability, and the possible aggravation of previously existing *grand mal* epilepsy. The minor side effects can be controlled easily by adjusting the dosage schedule, but the major side effects may necessitate withdrawal from the drug. It is apparent that JB-516 is a relatively safe drug for clinical use in pediatrics.

EFFECTS OF IPRONIAZID AND SIMILAR COMPOUNDS ON THE GASTROINTESTINAL TRACT

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It has been observed for the past 4 or 5 years that isoniazid and, recently, also iproniazidlike substances, administered for specific tubercular and mental disorders, tend to improve the appetite in certain instances. The increased appetite and consequent nutritional improvement and sense of well-being are salutary side effects. Consequently, internists and gastroenterologists have begun to use it particularly for its appetite-stimulating effect in those instances where appetite improvement was desired: in ulcerative colitis, in anorexia nervosa, and even in hepatitis and cirrhosis where the anorexia is a definite and marked symptom.

Over the years we have been impressed with the fact that about 60 per cent of these patients showed substantial gain in weight, general improvement in their nutritional status and, principally, a feeling of well-being. The doses we employed varied from 150 to 200 mg./day until we found that we could accomplish similar effects with amounts as small as 50 to 100 mg./day. It is noteworthy that, despite doses as large as 200 mg./day and the fact that iproniazid (Marsilid‡) was being used in cases of hepatitis and cirrhosis with liver damage, as well as in some old and debilitated patients, we have had only one case of jaundice and no deaths in 5 years. The one case of jaundice could not clearly be related to iproniazid, since the patient was simultaneously receiving a series of parenteral injections.

In the past 18 months we have observed our cases closely in the hope of finding the factor or factors that in those responsive cases might provide a clue to the basic physiological changes influencing the welfare of the patient.

The 61 patients chosen for iproniazid therapy in this study included some with symptoms of poor appetite due to hepatitis, cirrhosis, anorexia nervosa, poor nutritional state due to chronic duodenal ulcer, ulcerative colitis, geriatric wasting, and chronic or psychogenic nausea, as well as others who had little desire for food but were without definite evidence of organic disease (TABLE 1).

These patients ranged in age from 19 to 73 years and included 38 females and 23 males. They were carefully followed by laboratory studies in order to determine if any correlation could be found between the subjective symptoms of improved appetite, increased sense of well-being, and objective laboratory findings.

Of the patients under observation, 42 responded by subjective improvement after 3 weeks on Marsilid therapy, while 19 patients had no change in appetite, in improvement in their nutrition, or in sense of well-being.

Where the free and total HCl determinations revealed an increase in both groups, gastroscopy in some patients showed a marked hyperemia of the gastric mucosa, indicating some local irritating or sensitizing effect of iproniazid upon the mucosa. This was not observed in patients whose gastric acidity was not elevated.

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† Fellow, American College of Gastroenterology, and Fellow, American College of Physicians.

‡ Hoffmann-La Roche.

TABLE 1
PATIENTS SELECTED FOR IPRONIAZID THERAPY

Hepatitis	6
Cirrhosis	4
Anorexia nervosa	4
Chronic duodenal ulcer	12
Ulcerative colitis	8
Geriatric wasting	6
Nausea	14
No demonstrable organic disease	7
Total	61

TABLE 2
LABORATORY FINDINGS IN PATIENTS SHOWING SUBJECTIVE IMPROVEMENT*

	Increased	Decreased	Unchanged
Volume of gastric secretion	21	4	17
Free and total HCl	18	12	12
*p*H	17	10	15
Uropepsin	4	3	35
Liver function tests	3	0	39
Blood amylase	2	1	39
Blood sedimentation rate	4	2	36
Constipation	36	0	6
Weight	40	0	2

* The 19 nonresponsive patients showed similar inconsistencies of laboratory findings.

TABLE 3
LABORATORY FINDINGS IN PATIENTS SHOWING NO SUBJECTIVE IMPROVEMENT

	Increased	Decreased	Unchanged
Volume of gastric secretion	8	3	8
Free and total HCl	6	8	5
*p*H	7	6	6
Uropepsin	1	2	16
Liver function tests	0	0	19
Blood amylase	1	2	16
Blood sedimentation rate	2	1	16
Constipation	12	0	7
Weight	5	3	11

The over-all results were even more inconsistent when the laboratory data were compared case by case, and it was seen that patients who responded to iproniazid did not necessarily have parallel objective laboratory findings, but often showed effects found in patients without subjective improvement. Therefore we must conclude that, of the specific gastrointestinal changes observed, no single factor was consistently or significantly present or absent, except for constipation and weight gain, in either the group of 42 patients who responded or in the 19 who did not.

However, one consistent factor is of outstanding significance; namely, the presence of a definite hypoglycemia in patients whose appetite and sense of well-being improved. This improvement usually occurred after treatment with iproniazid for about 2 to 3 weeks and did not depend on the illness for which the medication was given. Surprisingly, even some patients with hepatitis showed this effect. When the drug was withdrawn the blood sugar rose to its normal level and could be depressed again by giving the patient the drug for an additional 2 to 3 weeks, during which time the symptoms of increased appetite and well-being reappeared (FIGURE 1).

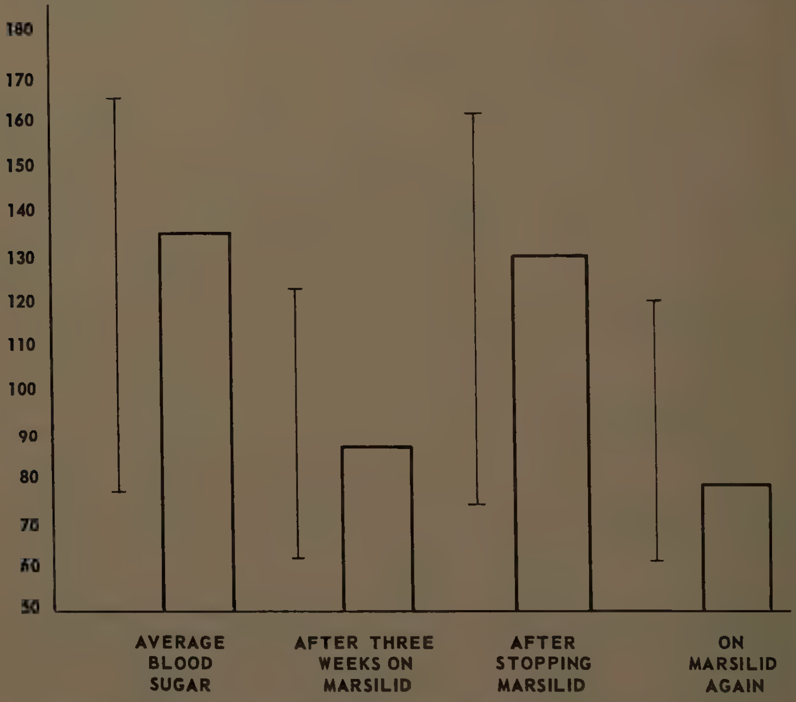

FIGURE 1. Composite blood sugar determinations in 42 responsive patients.

The combined averages of the blood sugar determinations of the 42 responsive patients were as follows:

Patients who did not report subjective improvement showed no change in their blood sugar levels.

The figures are significant in that they might, for the first time, indicate why these patients show an increased appetite, for it is well known that patients who exhibit hypoglycemia have marked hunger. This condition, in turn, leads to a greater food intake, with subsequent weight gain and, possibly, even a brighter outlook on life, which might even influence the patients' sense of well-being.

Each of these 61 patients was subjected to galactose tolerance tests: 40 gm. of pure galactose was ingested orally, and blood sugar determinations were made at periodic intervals. This test was repeated within 3 to 6 weeks after iproniazid medication, and it was noted that in every case there was a difference of 40 to 80 mg. between the normal curves and the curves of those patients who showed the hypoglycemic phenomenon. The average difference was 50 mg., and the structure of the curves was almost identical in all cases (FIGURE 2).

TABLE 4
BLOOD GLUCOSE IN MG. PER CENT OF 42 RESPONSIVE SUBJECTS

	Fasting blood sugar	After 3 weeks of therapy	3 weeks after withdrawal of drug	3 weeks after reinstitution of drug
Range	76 to 164	62 to 121	74 to 160	61 to 117
Average	134	86	128	76

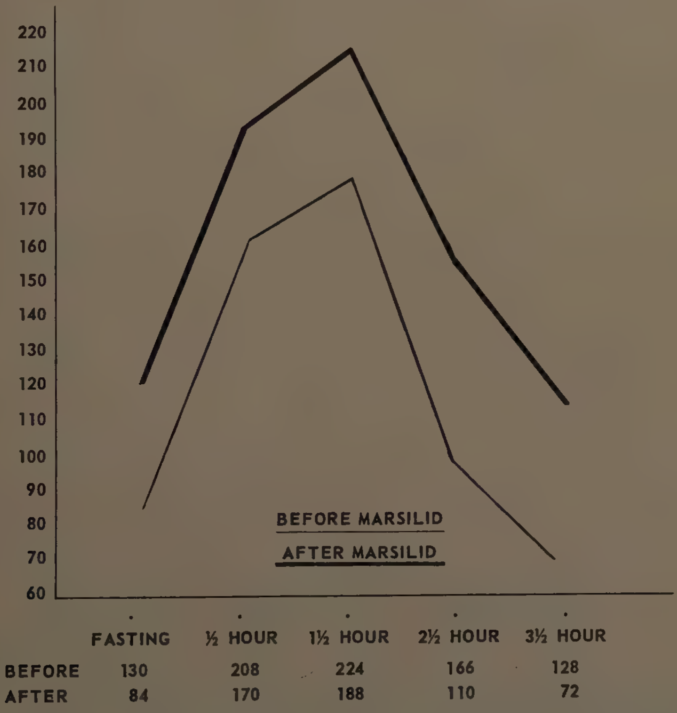

FIGURE 2. Composite galactose tolerance tests before and after the administration of Marsilid.

This tendency toward hypoglycemia was followed further in several patients by means of the insulin tolerance test. Each patient was given 5 units of regular insulin intravenously, and the blood sugars were checked at intervals. The results in 5 patients were compared by a similar procedure after the administration of iproniazid for 3 to 4 weeks and when these patients demonstrated both subjective signs and hypoglycemia. This time, however, the curve of the patients on iproniazid did not parallel the normal curve, but showed a delayed terminal rise (FIGURE 3).

This effect of iproniazid might be suggestive of hyperinsulinism, and could conceivably mean that the pancreas is the direct site of an action such as that of tolbutamide (Orinase) or insulin. Other suggestions are that this might be part of the effect that iproniazid exerts on the liver mechanism in altering glucose and glycogen metabolism or, possibly, an indirect effect of iproniazid which, by blocking amine oxidase, causes accumulation of norepinephrine and serotonin, with its resultant bizarre phenomena.

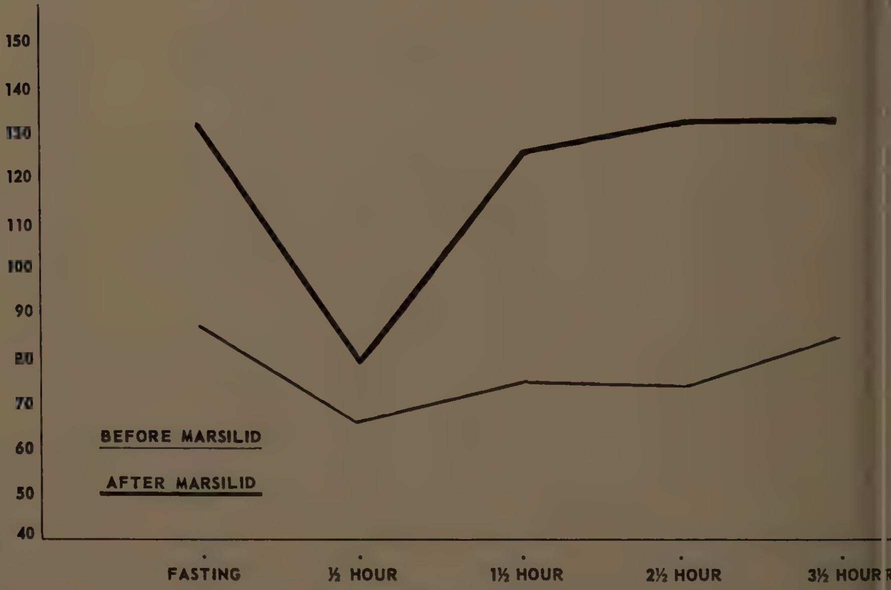

FIGURE 3. Composite insulin tolerance test.

We have also been doing clinical work along the same lines with 2 analogues, RO 4-1018 and RO 5-0700, and another substance, RO 2-6797/5. However, the cases are too few to warrant any conclusions. No definite trend has yet been observed except with RO 2-6797/5, in the use of which a marked rise in alertness and activity has been accompanied by a small drop in the blood sugar. No other physiological or pathological changes in the gastrointestinal tract or blood chemistries show any trend as yet.

Summary

We have presented evidence showing that about 69 per cent of patients respond to iproniazid therapy by exhibiting an increased appetite, better nutrition, weight gain, and improved sense of well-being, while mild constipation is a side effect of this form of therapy.

No consistent changes in gastric secretion, free acid, *p*H, and uropepsin could be demonstrated either in the patients who did respond or those who did not; most liver and pancreatic tests showed similar inconsistencies.

Where there was an increase in gastric acidity among patients from both groups, gastroscopy revealed evidence of a local irritating effect on the gastric mucosa.

The only significant and outstanding laboratory finding was a definite hypoglycemia that correlated consistently with a favorable subjective response to iproniazid and could be made to appear or recede by either administering or withholding the drug.

It is postulated that this phenomenon is probably due to a state of hyperinsulinism, the cause of which has yet to be established definitely. More work must be done along these lines if we are to determine the various specific physiological pathways and mechanisms whereby iproniazid and similar substances bring about the desired effects.

SOME OBSERVATIONS ON THE USE OF IPRONIAZID IN THE MANAGEMENT OF CRANIOCEREBRAL TRAUMA

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During the past 18 months a small group of patients with organic neurological disease has been treated with iproniazid, or Marsilid* (1-isonicotinyl-2-isopropylhydrazine). No attempt will be made to analyze the entire series, since conclusions arrived at from so small a number might well prove to be spurious. However, certain findings were noted in postoperative neurosurgical cases that seem worth reporting at this time.

In 1946 Bornstein[1] reported that free acetylcholine had been recovered in the cerebrospinal fluid (CSF) of animals subjected to cerebral concussion. Atropine was administered to these animals and appeared to provide a salutary response. Ward[2] reasoned from this that atropine might be useful in the management of humans who had suffered closed head injuries and indicated that a number of his patients who had sustained craniocerebral trauma were improved dramatically by atropine in very large doses; certain patients who were in decerebrate rigidity became alert and responsive but, if the atropine administration was not repeated, some patients again lapsed into a state of unresponsiveness. Sachs[3] later reported that in a series of patients with neurological disease a considerable number had free serotonin and/or free acetylcholine present in the CSF; as the disease process became stabilized in his patients the serotonin and acetylcholine disappeared from the CSF. Ruge[4] concluded from his experimental studies that atropine was effective in head trauma because of its blocking action on the muscarinic responses of acetylcholine. Tower and McEachern[5] had reported that acetylcholine was present in the CSF in large amounts in severe head injuries and that there was concomitantly low CSF cholinesterase activity. Foltz *et al.*[6] stated that preliminary histochemical studies performed by them did not reveal significant alteration in the cholinesterase concentration.

Clinical Material

A number of the patients reported herein were operated upon because of complications arising from craniocerebral injuries. One of these, a 58-year-old female, had developed an acute subdural hematoma that was evacuated 5 hours after her injury. She remained in decerebrate rigidity for 2 weeks following surgery, at which time iproniazid was administered, 50 mg. being given through a nasogastric tube twice daily. One week after institution of this regime the patient responded first to verbal stimuli and, 6 weeks later, was able to walk out of the hospital without help. Iproniazid was discontinued after 30 days. During this time 100 mg. of pyridoxine was administered daily. During her convalescence she was extremely restless, often sleeping no more than 2 hours in 24. She also suffered extreme urinary frequency. Mytelase chloride (ambenonium chloride), a bisquaternary compound of 6 times the potency of neostigmine as a reversible inhibitor of acetylcholinesterase, not only relieved the frequency but also provided a calming effect that permitted the patient to sleep for 3 and 4 hours at a time. An electroencephalogram was not obtained during the acute phase of her illness but, 2 weeks after iproniazid had been withdrawn and while the patient was still hyperactive and restless, a tracing showed high potential synchronous rhythm averaging 7 to 8 per second. A later record was obtained to determine whether or not mytelase showed a discernible electrographic effect. Despite both subjective and objective improvement in the patient's behavioral responses, the drug did not affect the EEG. A clinical psychologist who examined this patient during the latter part of her convalescence could find no evidence of intellectual impairment.

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* Hoffmann-La Roche.

Two other patients who had been in decerebrate rigidity postoperatively likewise made complete or nearly complete recoveries; both are back at work. These are the 3 most impressive cases. A number of others have been treated with little or no improvement. None has been made worse.

It is obviously impossible to attribute a direct relationship between the institution of a monoamine oxidase inhibitor and improvement in the neurological status of the patients described above. Similarly, it is difficult if not impossible to establish controls in a series of patients with head injuries. It can only be said that physicians in attendance were deeply impressed with both the degree and rapidity of improvement with iproniazid.

Discussion

Extensive reviews have been published recently on the interrelationship of monoamine oxidase,[7] its inhibitors,[8] and serotonin.[9] It appears that monoamine oxidase has a definite role in the catabolism of epinephrine, norepinephrine, histamine, and serotonin. Serotonin and monoamine oxidase are found in highest concentration in the brain stem. Iproniazid, which probably acts as an antimetabolite, is an inhibitor of monoamine oxidase and seems to result in a redistribution of serotonin and norepinephrine in the organism.

Because acetylcholine is normally not present in the CSF and since this compound is detectable there in a certain proportion of head injuries, one would expect some change in intracellular cholinesterase activity. Tower and Elliott[10, 11] found that the bound acetylcholine content of tissue removed from narcotized animals and incubated *in vitro* increased much more rapidly than that from normal tissue; the opposite was true of tissue from human epileptogenic foci. This finding suggested to them that the acetylcholine-binding capacity of the tissue is a factor in depression or excitation of activity in the central nervous system. Obviously, changes in cellular permeability and ratio of bound:free acetylcholine are involved, but experimental data have not as yet been obtained to elucidate their nature.

Foltz and Schmidt[12] demonstrated that sensory evoked responses in the reticular formation, absent after acceleration concussion, often recovered with time. Such responses in the medial lemniscus after concussion were similar to those found in control animals. These investigators felt that loss of sensory activation of the reticular formation was in part responsible for the unresponsive state that frequently follows head injury. Funderburk and Case[13] reported that the response of an atropinized animal represented a paradox in terms of the arousal reaction; although the animal was quite awake, a hypersynchronous electroencephalogram was recorded. Electroencephalographic arousal is manifested in the rhinencephalon by the appearance of synchronous waves rather than a desynchronized tracing.[14] Moruzzi and Magoun[15] have demonstrated that repetitive excitation of the reticular activating system induces arousal in a sleeping animal. French[16] found that electric stimulation of a peripheral nerve produced arousal by triggering the reticular activating system and, through it, the reticulocortical projections. In an excellent summary of present-day knowledge concerning the reticular formation, French[17] suggests that a re-evaluation of the existence or nonexistence of "suppressor strips" is in order. He points out that these areas are in or near corticoreticular loci. One is tempted to speculate that the mechanism of atropine in counteracting the lowered level of responsiveness in concussion is through its action upon the corticoreticular projections of the reticular activating system.

Summary

A small group of patients with neurological disease has been treated with iproniazid, a monoamine oxidase inhibitor. Beneficial results were obtained in some patients who had suffered acute craniocerebral trauma. The use of atropine in closed head injuries suggests a common or complementary mode of action. Both of these drugs may exert their action primarily upon the reticular activating system or its corticoreticular projections.

Acknowledgment

The Marsilid used in these studies was supplied by Hoffmann-La Roche Inc., Nutley, N. J.

References

1. Bornstein, M. B. 1946. Presence and action of acetylcholine in experimental brain trauma. J. Neurophysiol. **9:** 349–366.
2. Ward, A. A., Jr. 1950. Atropine in the treatment of closed head injury. J. Neurosurg. **7:** 398–402.
3. Sachs, E., Jr. 1957. Acetylcholine and serotonin in the spinal fluid. J. Neurosurg. **14:** 22–27.
4. Ruge, D. 1954. The use of cholinergic blocking agents in the treatment of craniocerebral injuries. J. Neurosurg. **11:** 77–83.
5. Tower, D. B. & D. McEachern. 1948. Acetylcholine and neuronal activity in craniocerebral trauma. J. Clin. Invest. **27:** 558–559.
6. Foltz, E. L., R. P. Schmidt, L. B. Thomas & A. A. Ward, Jr. Studies on the physiologic basis of cerebral concussion. School of Aviation Med. U.S.A.F. Rept. No. 55-111. : 19.
7. Davison, A. N. 1958. Physiological role of monoamine oxidase. Physiol. Revs. **38:** 729–747.
8. Symposium on the Biochemical and Clinical Aspects of Marsilid and Other Monoamine Oxidase Inhibitors. 1958. J. Clin. Exptl. Psychopathol. Quart. Rev. Psychiat. & Neurol. Suppl. **19**(2): 1–186.
9. Page, I. H. 1958. Serotonin (5-hydroxytryptamine); the last four years. Physiol. Revs. **38:** 277–335.
10. Tower, D. B. & K. A. C. Elliott. 1952. Activity of acetylcholine system in human epileptogenic focus. J. Appl. Physiol. **4:** 669–676.
11. Tower, D. B. & K. A. C. Elliott. 1953. Experimental production and control of an abnormality in acetylcholine metabolism present in epileptogenic cortex. J. Appl. Physiol. **5:** 375–391.
12. Foltz, E. L. & R. P. Schmidt. 1956. The role of the reticular formation in the coma of head injury. J. Neurosurg. **13:** 145–154.

13. Funderburk, W. H. & T. J. Case. 1951. The effect of atropine on cortical potentials. EEG Clin. Neurophysiol. **3:** 213–223.
14. Green, J. D. & A. A. Arduini. 1954. Hippocampal electrical activity in arousal. J. Neurophysiol. **17:** 533–557.
15. Moruzzi, G. & H. W. Magoun. 1941. Brain stem reticular formation and activation of the electroencephalogram. EEG Clin. Neurophysiol. **1:** 455–473.
16. French, J. D., F. K. von Amerongen & H. W. Magoun. 1952. An activating system in the brain stem of monkey. A.M.A. Arch. Neurol. Psychiat. **68:** 577–590.
17. French, J. D. 1958. The reticular formation. J. Neurosurg. **15:** 97–115.

THE METABOLISM OF IPRONIAZID

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Adequate understanding of the mode of action of a drug requires insight into its metabolic fate and its interaction with specific metabolic mechanisms. The significance of such information is especially obvious in the case of iproniazid (Marsilid*) whose pharmacological action has been defined thus far mainly in biochemical terms, that is, as that of an enzyme inhibitor affecting important regulatory tissue constituents.

In addition to our general interest in drug absorption, distribution, and excretion, we desired to obtain, through the present study, clues to various specific problems of a biochemical nature that had arisen in the course of previous clinical and pharmacological investigations.

Many observations had suggested that small amounts of Marsilid or its degradation products might be retained in the tissues where, accumulating upon repeated administration, they would gradually reach critical levels. Such a process would explain, on the one hand, the observed slow onset of the clinical drug response and, on the other, the drug-induced phenomena that persist for days after the discontinuance of the drug. Furthermore, the question had arisen whether metabolites rather than intact Marsilid were responsible for some of the drug effects, such as toxicity. This speculation, in turn, led to the hypothesis that metabolic differences might be responsible for the considerable variation of the drug response, not only in different species, but also in individuals of the same species. In the pursuit of clues to these and other questions, our investigation was carried considerably beyond the limits originally set for it.

For the tracer experiments in various laboratory animals, we used Marsilid with C^{14} in the 2-isopropyl carbon, prepared by the method of Fox.[2] This appeared to offer the best chance of tracing the hydrazide moiety of the molecule, the fate of which was of primary interest. However, biologically, this label did not remain as firmly attached to the hydrazide group as was expected, yet in other respects the choice turned out to be fortunate. With the help of sensitive chemical methods such as the picryl chloride tests for hydrazides,[2] the silver nitrate test for reducing substances,[3] the colorimetric determination of Marsilid[4] and of both isonicotinic acid and Marsilid,[5] and the ultraviolet absorption test for the isonicotinyl group, it has been possible to supplement the picture obtained by isotope tracing alone.

The tissue distribution that resulted after a single intraperitoneal dose of 5 mg./kg. labeled Marsilid in mice is shown in FIGURE 1. Tissue levels reached activity maxima 4 hours after dosage, falling off thereafter to a plateau rather rapidly. Except for the threefold to fourfold higher liver values and somewhat high spleen levels, the concentrations in most of the individual tissues followed the blood levels closely.

Similar tissue data are shown for the rat in TABLE 1. In liver, kidney, and red cells a higher relative radioactivity appeared to build up with time. We cannot go here into the apparent correlation of this finding with the well-known Marsilid effect on the red cell picture. The investigation of the nature of the substances responsible for the radioactivity of the tissues was difficult because, for the most part, they were firmly bound and not readily extracted under conditions that at the same time prevented the formation of artifacts. The small portion of extractable material showed a fairly complex chromatographic pattern. However, some intact Marsilid was still present 12 hours after administration and was excreted intact after that time.

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* Hoffmann-La Roche.

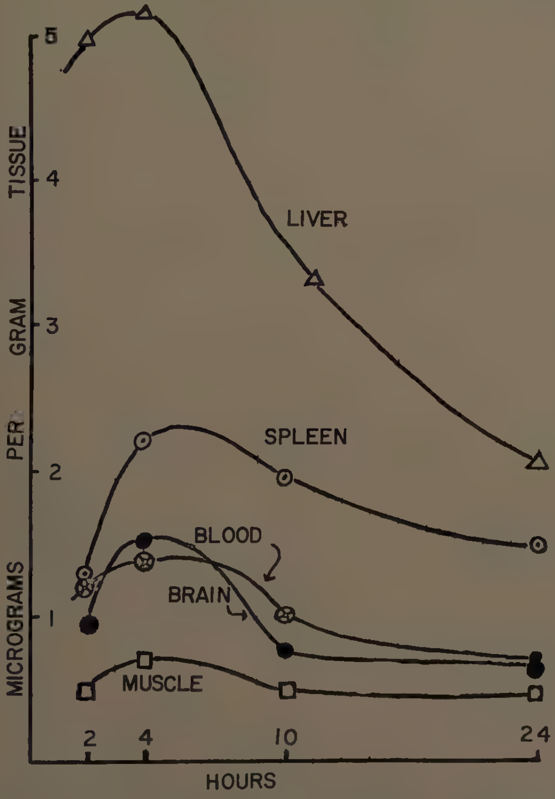


FIGURE 1. Tissue distribution in mice of radioactivity derived from C^{14}-Marsilid after the intraperitoneal administration of 5 mg./kg.

In rats and mice, about 15 per cent of the radioactivity, with a half life of about 3 hours, was excreted in the urine. The radioactive urinary excretion products exhibited a characteristic electrophoretic pattern that was similar in mice, rats, and rabbits. A typical electrophoretic separation is presented in FIGURE 2. FIGURE 3 represents a radioautograph of a 2-dimensionally developed urine sample, the abscissa denoting paper electrophoretic separation and the ordinate, chromatographic separation. One component represents intact Marsilid, accounting for about 5 per cent of the dose, and is designated by an arrow. Furthermore, 2 weakly basic metabolites and 1 major acidic one were recognized; these have since been isolated, and will be discussed later.

Table 1
Plasma and Tissue Concentrations of C^{14}-Marsilid in Rats
(Dose: 5 mg./kg.)

Tissue*	Results	2 hours	4 hours	6 hours
Plasma	μg. per ml.	2.15	1.22	0.78
Brain		0.84	0.66	
Carcass		0.82	0.93	
Kidneys		1.70	2.25	4.20
Spleen		0.75	1.02	1.62
Liver		2.90	3.20	4.80
Red Cells		1.10	1.35	2.55

* For brain and other tissues the results are expressed as tissue concentration over plasma concentration.

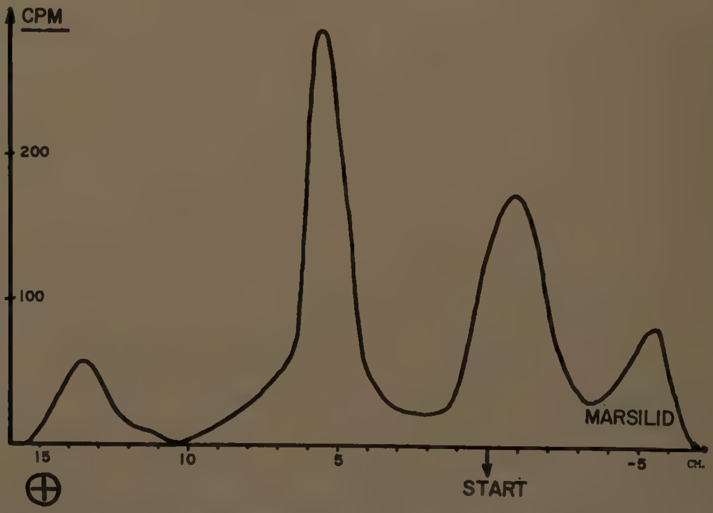
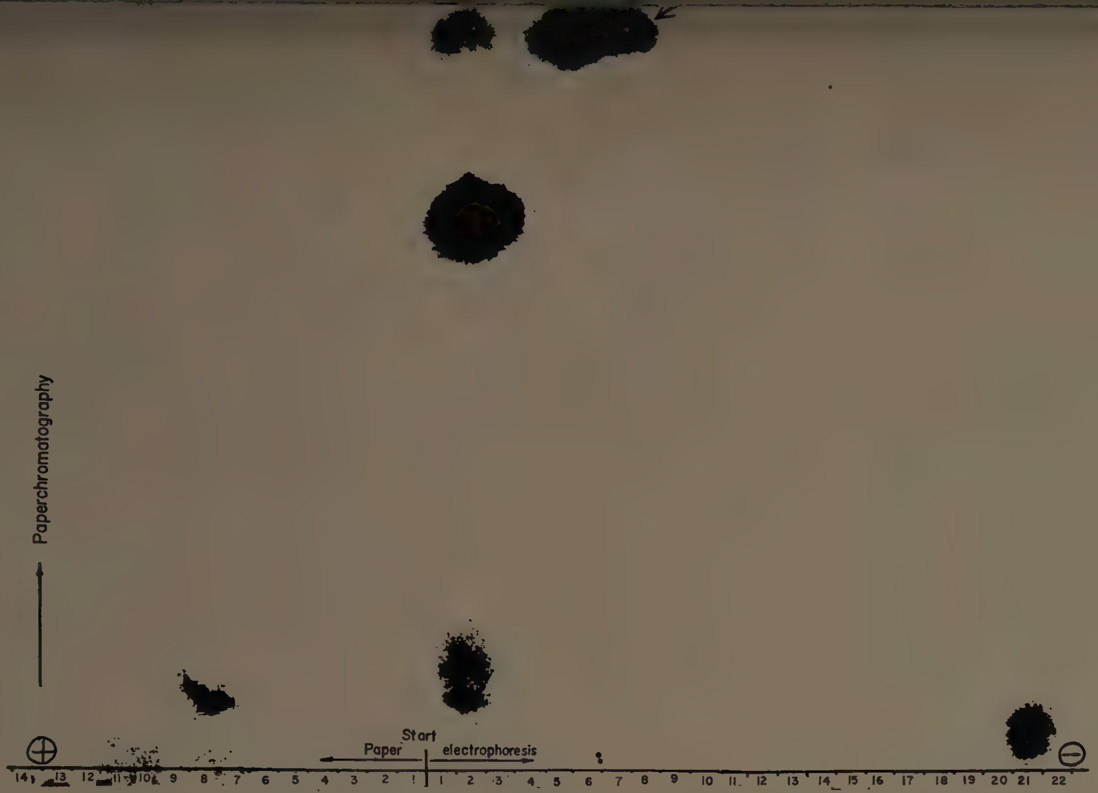


Figure 2. Electrophoretic pattern of mouse urine metabolites. Paper electrophoresis was carried out at 7 volts/cm.^{2} for 6.5 hours. The buffer used was citrate-phosphate, 0.5 M., *p*H 3.7. The total radioactivity applied to the Whatman No. 1 paper was 7000 cpm.

Ten per cent of the dose was found to be excreted into the stomach as Marsilid and to be partly reabsorbed from the intestinal tract.

The most important finding was that the major portion of the administered radioactivity was given off in the form of labeled CO_2 in the expired air. After a 5 mg./kg. dose in all 3 species, this portion accounted for at least 60 per cent of the C^{14} administered. Figure 4 shows that the rate at which the radioactive CO_2 is given off varies from species to species, the half lives for mice being near 2½ hours and for rats and rabbits, closer to 6 hours. More-



AUTORADIOGRAM OF RAT URINE METABOLITES

FIGURE 3. Autoradiogram of rat urine metabolites. The total radioactivity applied to the paper was 7500 cpm. First, paper electrophoresis was instituted under the same conditions as those listed under FIGURE 2. Next, paper chromatography was employed perpendicular to the direction of the electrophoresis. The solvent system used was butanol saturated with water. Radioautography was accomplished by exposing the paper to Kodak No-Screen X-Ray film for 97 days. The arrow (*upper center*) indicates the location of Marsilid.

over, it is interesting to note that these rates varied within the same species, that is, in mice from 1 to 3 hours and in rats from 4 to 7 hours.

We must expect that the formation of larger amounts of labeled CO_2 will also lead to the biosynthesis of radioactive proteins and lipids. Since these will gradually build up measurable radioactivity levels in the tissues, it will become difficult to differentiate this radioactivity from that of Marsilid and its immediate metabolites. Thus, this finding led to a reorientation of our investigation toward a detailed study of the metabolic breakdown reactions and their sequences.

The formation of radioactive CO_2 indicated ready cleavage and oxidation of the N_2-attached isopropyl side chain, a reaction to which we shall refer as oxidative N_2-dealkylation. It was subsequently found that acetone is an intermediate of this reaction. Labeled acetone was trapped and counted a the dinitrophenylhydrazone and was found in both expired air and urine.

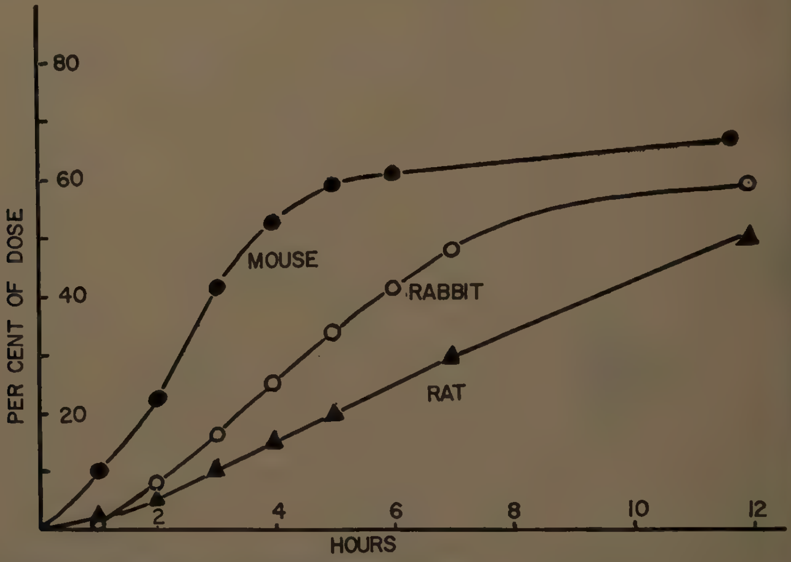


FIGURE 4. Expiration of radioactive CO_2 following the intraperitoneal injection of mg./kg. C^{14}-Marsilid in the mouse, rabbit, and rat.

On the other hand, by the isolation and identification of isonicotinic aci from urines of Marsilid-treated rats we were able to show that hydrolyti cleavage at the carbonyl group can occur biochemically.

It thus became apparent that, depending on the relative precedence an rates of these two major reactions, different major metabolic intermediate would be formed. Upon inspection of the scheme outlined in FIGURE 5 i is seen that, in the first case, isoniazid would be the primary metabolite and in the second case, isopropylhydrazine, which is itself not only a potent mono amine oxidase inhibitor, but also a potentially toxic compound. A thir possible reaction is the simultaneous formation of isonicotinic acid and acetone this is known to occur *in vitro* where Marsilid is autoxidized in slightly alka line media or in the presence of certain catalytic factors.

The breakdown mechanism could be investigated most conveniently in rats by administering high doses (200 mg./kg.). This technique made it possible to isolate urinary metabolites in sufficiently large amounts for chemical characterization by chromatographic, electrophoretic, spectrophotometric, and specific colorimetric techniques. In addition, by using an excessively high dose, a strain was put on the particular metabolic system. By making these enzymes work at near capacity, the possibility of the accumulation of metabolic intermediates was enhanced.

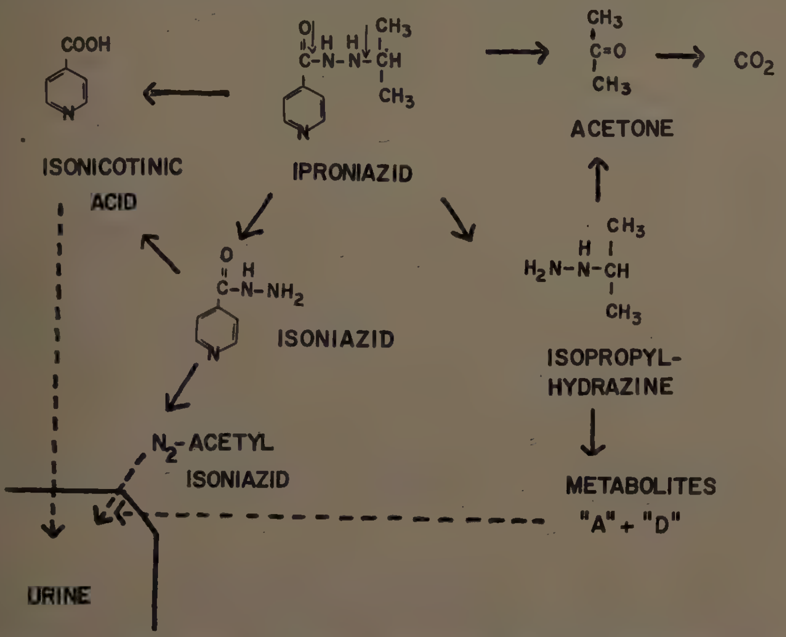


FIGURE 5. Scheme showing the possible reactions involved in the metabolism of Marsilid. The dot above the isopropyl carbon atom in iproniazid (Marsilid) denotes that this is the tagged atom. The dotted lines leading from isonicotinic acid, N_2-acetyl isoniazid, and metabolites "A" and "D" to the urine indicate that these compounds have been detected in the urine. Other compounds that have been shown to be products of the breakdown of Marsilid are acetone and carbon dioxide.

Examination of the urines so obtained revealed the excretion of small amounts of N_2-acetyl isoniazid, which was identified chromatographically and by its spectrum. On the other hand, no such direct conclusive evidence for the presence of either isopropylhydrazine or of its acetyl derivative could be obtained.

It was necessary to use a different, indirect approach to demonstrate the precedence of the hydrolysis at the carbonyl group and intermediate formation of isopropylhydrazine. By following the excretion rates of the total radioactivity and comparing it with those of total isonicotinic acid over a 24-hour period it was found, after a 50 to 200 mg./kg. dose, that the isonicotinic acid moiety of the molecule was excreted considerably faster than the radioactive portion (FIGURE 6). Since, according to Price and Rittenberg,[6] the half life of acetone in the rat is equal to or shorter than that of isonicotinic acid (about 2 hours), this finding provided evidence that the retained radio-activity represented an intermediate preceding the step of conversion to acetone, hence, isopropylhydrazine or a compound "on the level" of it.

More recently we have obtained additional evidence from the isolation of some of the urinary metabolites mentioned above. They are radioactive and contain the hydrazine group, but do not contain the isonicotinic acid portion of the Marsilid molecule. The properties of these metabolites, which are always found in the urine independent of the size of the dose, suggest that they represent condensation products of isopropylhydrazine with certain normal tissue constituents.

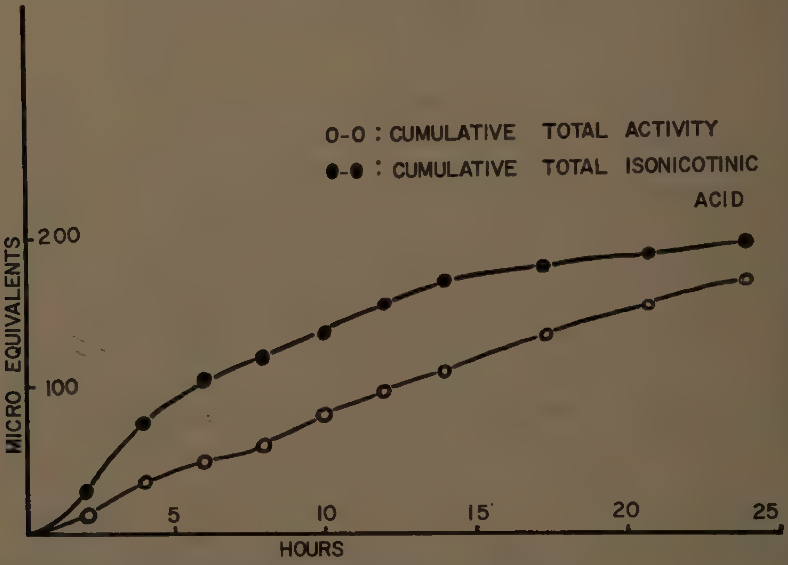


FIGURE 6. The cumulative excretion of isonicotinic acid and total radioactivity in the rat after an intraperitoneal dose of 200 mg./kg. of labeled Marsilid.

In FIGURE 7 the sum of the rates of acetone and CO_2 excretion are plotted as a function of time. Since both CO_2 and acetone possess relatively short half lives in the rat, we can regard the sum of these 2 values as an approximate measure of the rate of the oxidative N_2-dealkylation reaction and its changes over this 24-hour period. If we compare this rate after a 200-mg./kg. Marsilid dose with that reached after a 50-mg./kg. dose, which is included in this graph, it becomes apparent that this reaction step is inhibited by the high dose of Marsilid. This inhibition is, however, reversible and is usually overcome after 6 to 12 hours. It is significant that this reversal coincides, as shown in this figure, with the completed elimination of a large portion of the drug by urinary excretion, indicating that one or more of these metabolites inhibits the production of acetone plus CO_2.

The fact that this inhibition is reversible indicates that monoamine oxidase is not essential to the hydrazine dealkylating mechanism, since this enzyme is blocked irreversibly by Marsilid. Furthermore, it was not possible to block this metabolic step by repeated pretreatment with Marsilid.

A further important observation is that Marsilid at increased dosage levels inhibits the oxidation of acetone to CO_2. Table 2 shows that the portion of the total expired radioactivity present as acetone is about 3 times that which is oxidized to CO_2 after a 200-mg./kg. dose while, at lower Marsilid doses, the reverse relationship is found. This indicates that Marsilid interferes with one of the basic steps of intermediary oxidative metabolism.

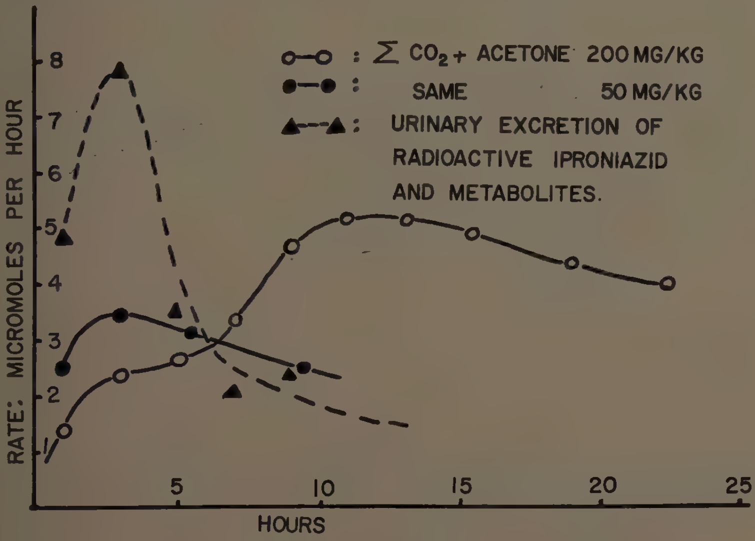


Figure 7. The sum of the rates of exhalation of labeled acetone plus carbon dioxide after a 50 mg./kg. dose and a 200 mg./kg. dose of labeled Marsilid and the urinary excretion of radioactive Marsilid and metabolites after the latter dose are presented as a function of time.

Table 2

Percentage of Total Expired Radioactivity in Rats Present as Carbon Dioxide and Acetone

Compound administered	Dose mM/kg.	Carbon dioxide	Acetone
Acetone*	0.15	90	10
	0.6	70	30
Iproniazid	1.0	27	73
	1.0	25	75
	0.25	50	50
	0.25	75	25
	0.03	80	20

* The acetone oxidation values were calculated from the data of Price and Rittenberg.[6]

This finding appears to be most interesting, since it draws our attention to a possible new major type of Marsilid action worthy of further study.

Acknowledgments

We acknowledge the important contributions made to this investigation by our collaborators W. Oberhansli, W. Kern, and their assistants, G. Tryon and E. Postma.

References

1. Fox, H. H. 1953. Synthetic Tuberculostats. VII. Monoalkyl derivatives of isonicotinylhydrazine. J. Org. Chem. **18:** 994.
2. Cuthbertson, W. F. J. & D. M. Ireland. 1952. The detection and identification of isonicotinic acid hydrazide and other tertiary pyridine derivatives. Biochem. J. **52:** XXXIV.
3. Wallenfels, K., E. Bernt & G. Limberg. 1953. Quantitative determination of reducing sugars by paper chromatography as well as an application for the microdetermination of blood sugar. Angew. Chem. **65:** 581.
4. Colarusso, R. J., M. Schmall, E. G. Wollish & E. G. E. Shafer. 1958. Photometric determination of iproniazid and related compounds. Anal. Chem. **30:** 62.
5. Rubin, S. H., L. Drekter, J. Scheiner & E. De Ritter. 1952. Determination of blood plasma levels of hydrazine derivatives of isonicotinic acid. Diseases of Chest. **21:** 439.
6. Price, T. D. & D. Rittenberg. 1950. The metabolism of acetone. I. Gross aspects of catabolism and excretion. J. Biol. Chem. **185:** 449.

EXPERIMENTAL PATHOLOGY OF IPRONIAZID AND RELATED COMPOUNDS

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Many hydrazine derivatives have monoamine oxidase (MAO) inhibiting activity *in vivo* that is often as strong as or even markedly stronger than that exerted by iproniazid (Marsilid*). Other compounds of the same chemical type exhibit only a weak MAO inhibition or none. These observations suggest that there are differences in absorption, breakdown, organ distribution, or detoxification of these drugs that may contribute to the variation in their biochemical properties. In the first part of this paper it will be shown that hydrazides and hydrazines of a similar type, all of which inhibit MAO very strongly, may lead to different organ changes when given in relatively high doses. This study was initiated in order to select new MAO inhibitors with fewer side effects for clinical use.

Methods

The compounds were selected from among the more than 300 MAO inhibitors synthesized in our chemical laboratories. Their laboratory numbers and chemical names are listed in TABLE 1. The drugs were given orally to groups of 4 rabbits each 5 times a week for 4 weeks. The dosage was equimolar with 50 mg./kg. and 10 mg./kg. iproniazid phosphate. Hemoglobin determinations and blood cell counts were performed before, in the middle, and at the end of the experiment. Twenty-four hours after the last administration of the substances the rabbits were sacrificed, and specimens of several organs were prepared for histological examination.

The same compounds were mixed in the diets of 30 to 35-day-old Wistar rats. The dosage was equimolar with 0.5, 0.25, and 0.1 per cent of iproniazid phosphate. Groups of 15 male animals were used for each dose. The composition for the basic diet is shown in TABLE 2.

After 2 weeks of treatment 3 to 5 animals of each group were sacrificed. Frozen sections of the sciatic nerves were colored with Sudan black B and Fat red 7B, and the extent of myelin sheath degeneration was estimated microscopically, as outlined in previous publications.[1, 2] The remaining animals were treated for 10 to 12 weeks more. At the end of the experiment, all surviving rats were sacrificed for histological examinations. Larger groups of untreated animals were included as controls in the rabbit and rat experiments.

Results

As expected, the high doses of all hydrazine compounds used in these experiments provoked similar blood and organ changes. There were, however, marked differences in the extent of the alterations, which are summarized in the following paragraphs. Some additional experiments designed to provide more information on the effects observed will also be briefly mentioned.

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General toxicity. The general toxicity of the MAO inhibitors tested varies over a very wide range. Iproniazid is tolerated by rabbits in an oral dose of

TABLE 1

LABORATORY DESIGNATIONS AND CHEMICAL NAMES OF THE COMPOUNDS TESTED

RO 4-0997 = N^1-[2-Methoxy-isonicotinyl]-N^2-isopropylhydrazine
RO 4-1018 = N^1-[DL-N-Acetyl-methionyl]-N^2-isopropylhydrazine
RO 4-1027 = N^1-[p-Chlorobenzoyl]-N^2-isopropylhydrazine
RO 4-1038/1 = DL-Serine N^2-isopropylhydrazide
RO 4-1107 = N^1-[α-Hydroxypropionyl]-N^2-isopropylhydrazine
RO 4-1108 = N^1-[2-Aminobenzoyl]-N^2-isopropylhydrazine
RO 4-1340 = L-α-Alanine N^2-isopropylhydrazide
RO 4-1343 = N^1-Acetyl-N^1, N^2-di-isopropylhydrazine
RO 4-1355/1 = N^1-[2-Thenoyl]-N^2-isopropylhydrazine
RO 4-1385 = L-Glutaminic acid α-isopropylhydrazide
RO 4-1417 = L-Glutaminic acid γ-isopropylhydrazide
RO 4-1418 = L-(+)-Glutamine α-isopropylhydrazide
RO 4-1528 = N^1-[Pyridyl-4-methylene]-N^2-isopropylhydrazine
RO 4-1530 = 1,2-Diisopropylsemicarbazide
RO 4-1565/1 = L-Leucine β-isopropylhydrazide
RO 4-1634 = N^1-Pivaloyl-N^2-benzylhydrazine
RO 4-1679 = Diethoxyacetic-acid isopropylhydrazide
RO 4-1737 = Sarcosin β-isopropylhydrazide
RO 4-2068 = DL-Serine α-benzyl-ethylhydrazide
RO 2-7687 = N^1-Palmitoyl-N^2-isopropylhydrazine
RO 5-0700 = N^1-Picolinyl-N^2-benzylhydrazine
RO 5-0831 = N^1-[5-Methyl-3-isoxazolylcarbonyl]-N^2-benzylhydrazine
Marsilid = N^1-Isonicotinyl-N^2-isopropylhydrazine

TABLE 2

COMPOSITION OF BASIC DIET

Casein	20 per cent
Coconut oil	10 per cent
Sucrose	61 per cent
Salt mixture	4 per cent
Cellulose	5 per cent

Vitamins, daily dose per rat:

Thiamine	100 γ	Vitamin D_2	5 I.U.
Pyridoxine HCl	100 γ	Riboflavin	200 γ
Choline	3 mg.	(+)-Biotin	0.5 γ
Ca pantothenate	200 γ	Niacinamide	1 mg.
DL-α-Tocopherol	30 γ	2-Methyl-1,4-naphthoquinone	20 γ
		Vitamin A	15 I.U.

50 mg./kg., whereas other hydrazides given in equimolar doses were fatal to the animals in a few days; some were not even tolerated at one fifth of this dose. As may be seen in TABLE 3, the general toxicity of the MAO inhibitors is in some way related to the enzyme-inhibiting activity, as demonstrated earlier by Zeller and his co-workers.[3] There are, however, many exceptions to this rule; for example, the two compounds RO 5-0700 and RO 5-0831/1 are very strong MAO inhibitors, but are nevertheless well tolerated in high doses.

Effect on erythrocytes. It is well known that hydrazines and hydrazides have hemolytic properties and that phenylhydrazine has been used for destruction of erythrocytes in polycythemia vera. Large doses of most of the MAO inhibitors tested led to a decrease in hemoglobin and in the number of red cells in the blood of all animal species studied. Concurrently, an increase in the number of reticulocytes, as well as qualitative alterations of the red cells, including the formation of Heinz-Ehrlich bodies, could often be observed (FIGURE 1). Histological examination of the organs revealed other signs of increased erythrocyte destruction, such as hemosiderosis of the spleen, liver, and kidney, (FIGURE 2), increased cellularity of the bone marrow, and hyperemia of the spleen.[4] The experiments with rabbits showed that not all the MAO inhibitors tested had the same hemolytic properties when given in

TABLE 3

GENERAL TOXICITY AND HEMOLYTIC EFFECT OF VARIOUS MAO INHIBITORS IN RABBITS

Compound	MAO inhibition*	General toxicity†		Change of hemoglobin (mg. per cent)‡		Ave. number reticulocytes at end of experiment (per 1000)‡		Ave. number Heinz-Ehrlich bodies at end of experiment (per 1000)	
		HD§	LD**	HD	LD	HD	LD	HD	LD
RO 4-1018	+++	0	0	*−2.8*	−1.1	*165*	29	55	0
RO 4-1038/1	+++	0	0	*−4.0*	*−2.6*	*247*	*85*	71	0
RO 4-1108	+	0	0	−1.3	−0.5	60	37	0	0
RO 4-1340	+++	0	0	*−3.0*	−1.3	*128*	31	175	0
RO 4-1343	+++	++++	++++	—	—	—	—	—	—
RO 4-1355/1	+++	0	0	*−4.3*	*−2.1*	*203*	*107*	518	0
RO 4-1385	+++	0	0	*−2.6*	−0.8	††	††	††	††
RO 4-1417	+++	0	0	*−2.2*	*−2.1*	*135*	54	91	0
RO 4-1418	+++	0	0	*−3.9*	*−3.0*	*207*	57	35	0
RO 4-1528	+++	0	0	*−4.2*	−1.4	*135*	54	136	0
RO 4-1530	+++	++++	0	—	−1.4	—	54	—	0
RO 4-1565	+++	++++	+	—	−1.3	—	32	—	0
RO 4-1634	++	0	0	−0.7	−0.2	51	46	0	0
RO 4-1679	+++	+	0	*−4.4*	−1.5	*128*	41	427	0
RO 4-1737	++	0	0	−0.8	+0.4	40	31	0	0
RO 4-2068	++++	++++	0	—	−1.8	—	*68*	—	114
RO 2-7687	+	++	+	*−3.3*	−1.5	*85*	38	0	0
RO 5-0700	++++	0	0	*−2.2*	−0.2	*107*	37	15	0
RO 5-0831/1	++++	0	0	−1.5	+0.5	*87*	47	87	0
Marsilid	++	0	0	*−4.9*	−1.5	222	39	78	0
Controls (11)	0	0		−1.1 ± 0.8§§		43 ± 20§§		0	

* MAO inhibition in the brain of rats $\frac{\text{ED 100 iproniazid}}{\text{ED 100 compound}}$: <1 = +; = 1–2 = ++; 2–5 = +++; >5 = ++++.

** LD = Low dose, equimolar with 10 mg./kg. iproniazid phosphate.

† General toxicity: +–++++ = number of rabbits died during the experiment.

‡ Italicized figures denote statistical significance ($p < 0.05$).

§ HD = High dose, equimolar with 50 mg./kg. iproniazid phosphate.

†† Not determined.

§§ $SD = \sqrt{\frac{S(x - x)^2}{(n - 1)}}$

equimolar doses. As may be seen in TABLE 3, most of the compounds led to a lowering of hemoglobin content with reticulocytosis and formation of Heinz-Ehrlich bodies, especially at the higher doses. Some compounds, however, such as RO 4-1634, RO 4-1737, and RO 5-0831/1, had only a very slight effect, if any, on the red blood cells. The weak MAO inhibitor RO 4-1108 induced a moderate fall in the hemoglobin content in the first half of the experiment, followed by a regeneration during the second half. As may also be seen in TABLE 3, there is no clear relation between MAO-inhibiting activity or general toxicity and the hemolytic properties of the compounds.

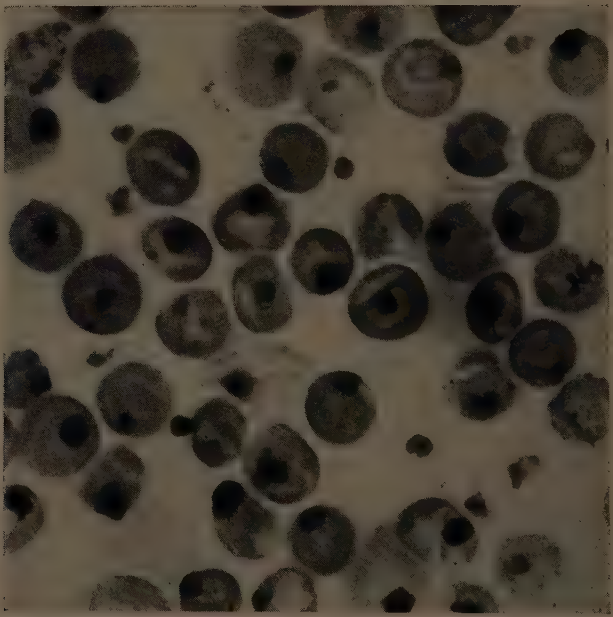

FIGURE 1. Blood smear, stained with brilliant cresyl blue, from a dog treated with 5 to 35 mg./kg. iproniazid (base) subcutaneously during 9 weeks. Note the formation of Heinz-Ehrlich bodies. ×1300.

To elucidate the effect of MAO inhibitors on red blood cells, iproniazid was given to groups of 4 rabbits each 5 times weekly in oral doses of 50, 10, and 5 mg./kg. The average lifetime of erythrocytes was measured by the radioactive chromium technique. FIGURE 3 shows that 50 mg./kg. and 10 mg./kg. led to a marked acceleration of erythrocyte breakdown, whereas 5 mg./kg. exhibited no hemolytic activity.

Effects on the liver. Some hydrazines are known to be hepatotoxic substances that cause fatty infiltration and necrosis of the liver. There are however, marked differences in hepatotoxicity among compounds of this class. Isoniazid, for example, is widely used in therapy of tuberculosis and shows practically no hepatotoxic side effects in humans. This compound, also very well tolerated by experimental animals, increases the fat content of the liver only if given in very high doses.[5] Among the MAO inhibitors tested thus

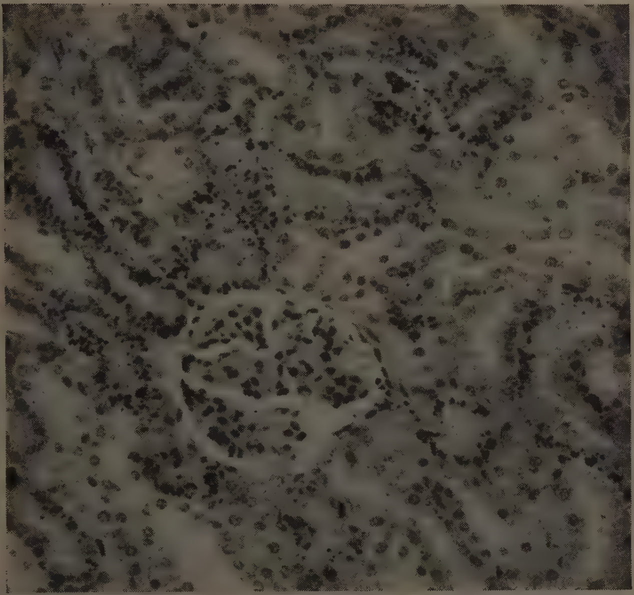

Figure 2. Kidney from same dog as in figure 1. Note hemosiderosis of the kidneys. Berlin blue reaction. ×250.

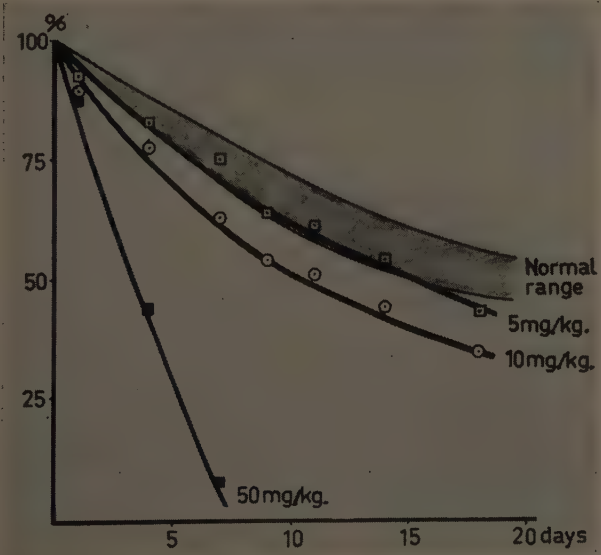

Figure 3. Average lifetime of erythrocytes in rabbits orally treated with iproniazid, measured by the radioactive chromium technique (average of 4 rabbits).

far on rabbits, 3 compounds have been found that induce severe swelling, fatty infiltration, and necrosis of the liver: RO 4-1343, RO 4-1565, and RO 4-1355/1. The liver damage was usually accompanied by some degenerative changes and fatty infiltration of the kidneys. The other compounds, including iproniazid, led at most to a slight-to-moderate fatty infiltration of liver cells, but not to liver necrosis. Hepatitislike changes in the liver, as reported by Popper[6] in several human cases of jaundice during treatment with iproniazid, could never be induced in experimental animals, even when sublethal doses were administered. This may be seen in the following example.

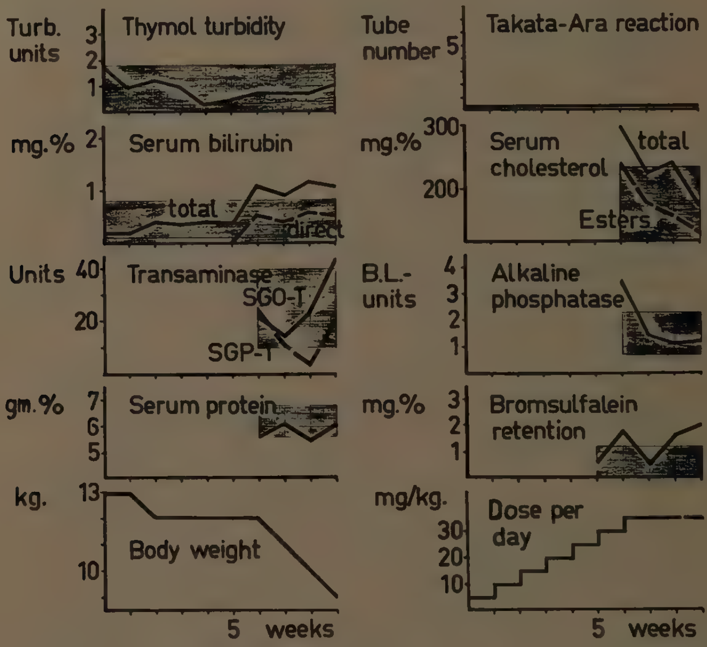

FIGURE 4. Liver function tests performed in a dog treated with subcutaneous injections of iproniazid (base). The same animal as in FIGURES 1, 2, and 5.

A dog received subcutaneous injections of iproniazid 5 times a week. The daily dose was 5 mg./kg. in the first week and was increased by 5 mg./kg. each week thereafter. The dog tolerated the drug up to 25 mg./kg. quite well. After 5 injections of 30 mg./kg., however, signs of toxicity appeared. A very slight increase in bilirubin, cholesterol, and alkaline phosphatase could be demonstrated, and bromsulphalein excretion was somewhat impaired. Although the general condition of the dog was very bad during the period of treatment with 35 mg./kg., liver function tests remained unchanged. Thymol turbidity and Takata-Ara reaction were normal. Transaminase activity in the serum reached the upper level of the normal range at the end of the experiment (FIGURE 4). Histological examination of the liver showed marked fatty infiltration, but no necrosis or inflammation (FIGURE 5).

From these investigations it can be seen that iproniazid by itself has little effect on liver function and structure, even when it is given in very high dosages. The question then arises as to whether the MAO inhibitors might intensify liver damage caused by other agents. To investigate this possibility, several forms of liver damage were produced experimentally and studied with and without iproniazid treatment. Liver changes induced by ethionine, phenylhydrazine, allyl alcohol, and the chicken hepatitis virus (R. J. Schnitzer, personal communication) were not enhanced by iproniazid. In another experiment, liver damage was produced in rats by injections of 0.3 mg./kg. of carbon tetrachloride. In normal rats this amount of carbon tetrachloride produced centrolobular swelling of liver cells, but usually no liver necrosis.

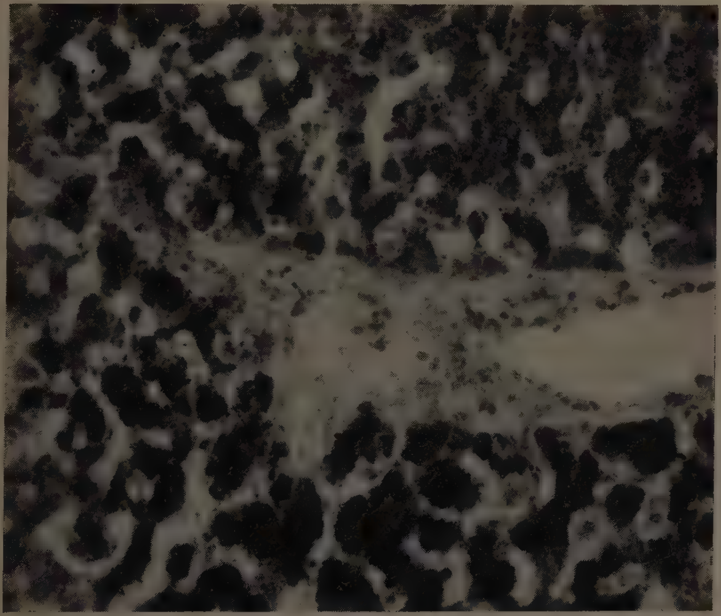

FIGURE 5. Liver of the same animal as in FIGURE 1, stained with Fat red. Note fatty infiltration of the liver cells. ×220.

In rats pretreated with 100 mg./kg. iproniazid daily for 15 days, however, the same dose of carbon tetrachloride produced centrolobular liver cell necrosis. As may be seen in FIGURE 6, a similar enhancement of carbon tetrachloride toxicity could also be demonstrated with other hydrazides, but not with all of them. Recent investigations have shown that the enhancement of carbon tetrachloride liver toxicity by some MAO inhibitors may also be demonstrated in rats by the determination of effects on serum transaminase levels (R. E. Bagdon, personal communication). These experiments indicate that some MAO inhibitors may aggravate the severity of certain liver diseases. Much more clinical work must be done, however, to find out whether the experimental findings have any significance for the evaluation of the side effects of the drugs in humans.

Effect on male reproductive organs. Impotence occurs rather frequently during therapy with MAO inhibitors in humans. We therefore studied the effect of these compounds on reproductive organs in male rats. Even after prolonged treatment with therapeutic doses of these substances, no changes were found in testicles, prostate, or seminal vesicles. After repeated administration of higher doses, however, slight-to-marked atrophy of the testicles could be seen with impairment of spermatogenesis, degeneration of germinal epithelium, and formation of giant cells.[4] As indicated in FIGURE 7, the effect of the various compounds is not uniform. Some of the MAO inhibitors, for

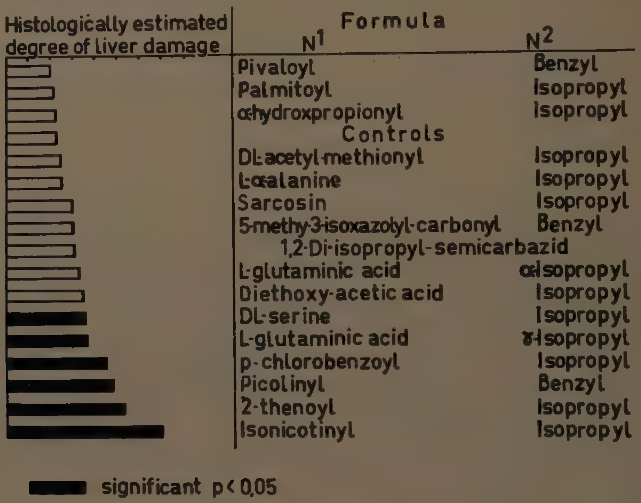

FIGURE 6. Enhancement of carbon tetrachloride liver toxicity by hydrazides in rats.

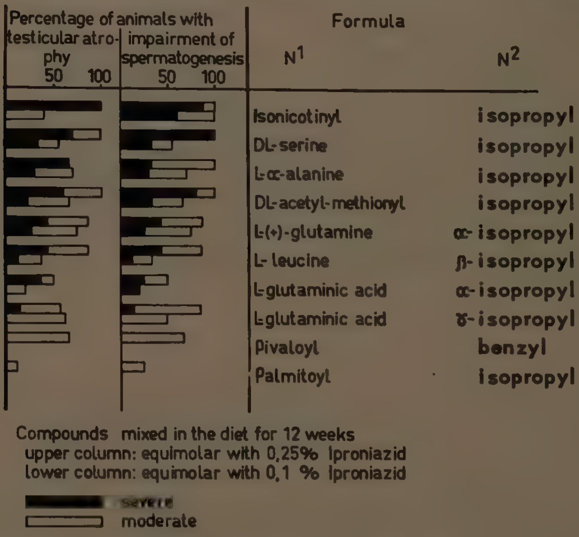

FIGURE 7. Effects of hydrazides on the testicles of young rats.

instance RO 4-1634, RO 2-7687, RO 4-1385, and RO 4-1417, have only a very slight and inconstant effect. Others, however, lead to more severe testicular changes when given in equimolar doses. No obvious relationship to the MAO-inhibiting activity of the compounds could be detected.

It is clear that the effect of MAO inhibitors on germinal epithelium cannot explain the decrease of libido observed in humans. The Leydig cells do not seem to be influenced by the drugs. In a 7-week experiment rats were treated with 0.1 per cent iproniazid in the diet (about 80 mg./kg./day). In contrast to a control group of castrated animals, the hydrazide-treated rats showed only a moderate atrophy of the prostate and seminal vesicles and no castration cells in the anterior pituitary lobe (TABLE 4). From this experiment it may be concluded that the impotence in patients treated with MAO inhibitors is more likely to be due to specific effects on central nervous centers than to a disturbance in the production or release of male sex hormone. In addition, it can be mentioned that no impairment of sexual activity was observed in iproniazid-treated mice.

TABLE 4

INFLUENCE OF IPRONIAZID ON REPRODUCTIVE ORGANS IN MALE RATS

Treatment 7 weeks	0.1% iproniazid in the diet	Controls	Castrated	Castrated + iproniazid
Number of rats	10	10	11	13
Body weight	169 ± 19	220 ± 25	210 ± 25	155 ± 15
Weight of prostate and seminal vesicle				
absolute (gm.)	0.83 ± 0.3	1.91 ± 0.21	0.15 ± 0.04	0.17 ± 0.09
relative %	0.49 ± 0.15	0.90 ± 0.12	0.07 ± 0.02	0.11 ± 0.05
Atrophy of testicle	+	0	—	—
Atrophy of prostate and seminal vesicle	+	0	+++	+++
Castration cells in ant. pituitary lobe	0	0	+++	+++

Effect on peripheral nerves. From clinical reports on the use of isoniazid in tuberculosis we know that peripheral neuritis is one of the most frequent side effects of this drug. If isoniazid is given in high doses to rats, severe degeneration of myelin sheaths is produced.[1, 2] Iproniazid, however, despite its close chemical relationship to isoniazid, causes only a very slight swelling or degeneration of myelin sheaths in rats when given in sublethal doses.[4] In accordance with this finding, peripheral neuritis is practically never encountered in iproniazid-treated patients. In the rat experiments (FIGURE 8) many compounds failed to affect the peripheral nerves at the doses tested. Some of them led to a slight degeneration of myelin sheaths. There is, however, one compound (RO 4-0977) that was highly active and definitely injured peripheral nerves even more than isoniazid did. This compound showed no particular general toxicity and it seems that the introduction of a methoxy group in the 2-position of iproniazid imparts to this substance a high affinity to neural tissue.

Discussion

Until now all compounds that are known to be strong and irreversible MAO inhibitors *in vivo* have a hydrazide or hydrazine structure.[7] It is probable that the blood and tissue changes that occur after administration of these drugs are primarily due to a direct action of the highly active NH-NH group rather than to MAO inhibition, since the extent of the toxic symptoms is usually not closely related to the enzyme-inhibiting activity. If the NH-NH moiety is considered to be responsible for the toxic quality of the compound, then the remainder of the molecule seems to determine the organ affinity. This could explain why certain hydrazides are characterized by marked hemolytic activity, whereas others are more injurious to the liver and kidneys, the peripheral nerves, or the germinal epithelium. Although the toxic effects observed in experimental animals are not directly comparable to the side effects that may occur during administration of therapeutic doses to patients, preliminary investigations have shown that MAO inhibitors with few toxic effects in animal experiments are also better tolerated by humans.

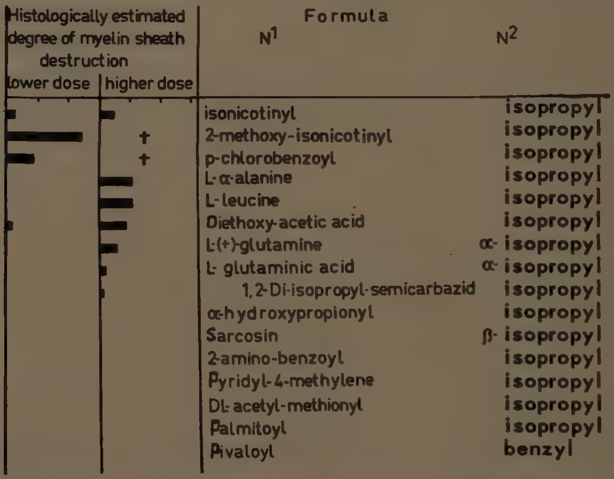

FIGURE 8. Damage to peripheral nerves in rats induced by the feeding of hydrazides for 15 days.

Histochemical and Histological Demonstrations of the MAO-Inhibiting Effects of Iproniazid

The biochemical effects of MAO inhibitors are chiefly demonstrated by measuring the level and distribution of monoamines in tissues. Serotonin and the catecholamines (CA) can also be demonstrated histochemically in the enterochromaffin cells of the intestine and the adrenal medulla, respectively.[8-10] After treatment with iproniazid the concentration of these amines increases in several organs;[11-13] however, this cannot be demonstrated by histochemical techniques. After a single injection of 5–10 mg./kg. of reserpine a release of serotonin and CA occurs that can be shown by the disappearance of the characteristic histochemical reactions in the enterochromaffin cells and the adrenal medulla.[9, 10, 14-18] Sixteen to 20 hours after pretreatment with 50 to 100 mg./kg. iproniazid, the reserpine-induced fading of the histochemical reactions is markedly reduced. Pretreatment with isoniazid has no effect.[10, 19] These observations may indicate that the binding of serotonin and CA on tissue structures is enhanced in some way when MAO is blocked. If the rats are injected with 30 mg./kg. harmaline intraperitoneally 1 hour prior to a single administration of 100 mg./kg. iproniazid, the histochemical CA reaction is considerably reduced by 5 to 10 mg./kg. reserpine given 16 to 20 hours after iproniazid. Harmaline is a very short-acting MAO inhibitor that blocks the MAO for only a few hours.[20] Iproniazid given during the period in which harmaline is active does not reach the enzyme,[21] is metabolized, and therefore gives no protection against the CA-releasing effect of reserpine. MAO inhibition may also be involved in the following phenomenon. Multiple gastric ulcers are produced in rats by feeding the animals sugar solution exclusively for 8 to 15 days. A daily treatment with 1 mg./kg. reserpine leads to a highly significant increase in the average number of gastric ulcers. This effect of reserpine is completely inhibited by injection of 50 mg./kg. of iproniazid 3 times a week. Rats which receive only iproniazid show even fewer gastric ulcers than the animals in the control group[22] (FIGURE 9). It is possible that a lowered serotonin content of the gastric mucosa in reserpine-treated animals facilitates ulcer formation, whereas the inhibition of serotonin released by iproniazid has a contrary effect.

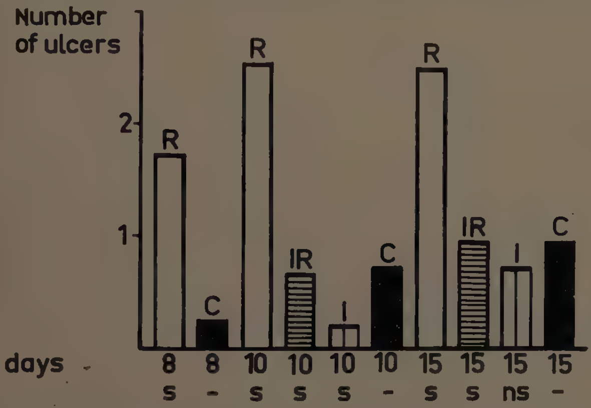

FIGURE 9. Gastric ulcers induced in rats by feeding sugar solution exclusively for 8, 10 or 15 days. Numbers of ulcers were counted in 6 different slides of the gastric mucosa of each animal. Groups of 12 rats were used. Symbols: s, significant; ns, not significant. Treatment: R, 1 mg./kg. reserpine intraperitoneally (I.P.) daily; I, 50 mg./kg. iproniazid I.P. 3 times weekly; IR, 1 mg./kg. reserpine I.P. daily and 50 mg./kg. iproniazid I.P. 3 times weekly; C, control animals.

Summary

Experimental data are presented indicating that iproniazid and other MAO inhibitors exhibit a direct toxic effect on various organs if they are given in high doses to experimental animals. These effects are probably due to the hydrazide or hydrazine moiety, whereas the extent of the alterations and the

affinity to certain organs seem to be dependent on the substituents. Other experiments show that iproniazid can also lead indirectly to organ alterations by its enzyme-inhibiting property.

References

1. Zbinden, G. & A. Studer. 1955. Schweiz. Z. allg. Pathol. **18:** 1198.
2. Zbinden, G. & A. Studer. 1955. Z. Tuberk. **107:** 97.
3. Zeller, E. A., J. Barsky, E. R. Berman & J. R. Fouts. 1954. J. Allergy **25:** 69.
4. Zbinden, G. Erg. allg. Path. **39:** In press.
5. McKennis, H., A. S. Yard & E. V. Panhelas. 1956. Am. Rev. Tuberc. **73:** 956.
6. Popper, H. 1958. J. Am. Med. Assoc. **168:** 2235.
7. Zeller, P., A. Pletscher, K. F. Gey, H. Gutmann, B. Hegedüs & O. Straub. 1959. Ann. N. Y. Acad. Sci. **80**(3): 555.
8. Zbinden, G. & A. Studer. 1958. Tagg. Deut. ges. pathol. **42:** 22–26.
9. Zbinden, G., A. Pletscher & A. Studer. 1957. Schweiz. med. Wochschr. **87:** 629.
10. Zbinden, G. & A. Studer. 1958. Experientia. **14:** 201.

11a. Pletscher, A. 1956. Experientia. **12:** 479.
11b. Pletscher, A. 1956. Helv. Physiol. Acta. **14:** C76.
12a. Udenfriend, S., H. Weissbach & D. F. Bogdanski. 1957. Ann. N. Y. Acad. Sci. **66**(3): 602.
12b. Udenfriend, S., H. Weissbach & D. F. Bogdanski. 1957. J. Pharmacol. Exptl. Therap. **120:** 255.
13. Spector, S., D. Prockop, P. A. Shore & B. B. Brodie. 1958. J. Pharmacol. Exptl. Therap. **122:** 71A.
14. Vialli, M. & E. Quaroni. 1956. Riv. istochim. norm. e patol. **11:** 111.
15. Benditt, E. P. & R. L. Wong. 1956. Am. J. Pathol. **32:** 638.
15a. Benditt, E. P. & R. L. Wong. 1957. J. Exptl. Med. **105:** 509.
16. Marks, B. H., R. W. Sorgen & E. R. Hayes. 1958. Proc. Soc. Exptl. Biol. Med. **97:** 413.
17. Camanni, F., O. Losana & G. M. Molinatti. 1958. Experientia. **14:** 199.
18. Eränkö, O. & V. Hopsu. 1958. Endocrinology. **62:** 15.
19. Zbinden, G., A. Pletscher & A. Studer. 1957. Klin. Wochschr. **35:** 565.
20. Udenfriend, S. & H. Weissbach. 1958. Proc. Soc. Exptl. Biol. Med. **97:** 748.
21. Pletscher, A. & H. Besendorf. Experientia. In press.
22. Zbinden, G., A. Pletscher & A. Studer. 1959. Schweiz. med. Wochschr. **89:** 289.

THE RELATIONSHIP OF CERTAIN VITAMIN DEFICIENCIES TO THE TOXICITY OF IPRONIAZID

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Biochemical studies of iproniazid (Marsilid*) have been prompted by the need to know more about the mechanism of its action and the nature of its toxicity. Since the structure of isonicotinic acid hydrazide (INH) and iproniazid suggest a relationship to two B vitamins, pyridoxine and niacin, it was decided to ascertain what, if any, relationships existed between certain vitamin deficiencies and the toxicity of iproniazid. The chronic and acute toxicity of this drug was determined in rats and in mice of different strains maintained on purified diets deficient in pyridoxine, niacin, or pantothenic acid. In addition, the effect of iproniazid, INH, and isopropyl hydrazine (IPH) on the activities of three enzymes that require pyridoxal phosphate as a cofactor was determined in brain and liver.

The toxic effects of such compounds may be more readily apparent under conditions of stress and may be exaggerated by a nutritional deficiency. For example, the toxicity of INH was much greater in pyridoxine-deficient rats[1, 2] and INH-inhibited tryptophanase[3] and arginine decarboxylase,[4] which require pyridoxal phosphate as a coenzyme. Also, the toxicity of a pyrimidine, which was studied as an antimetabolite of pyridoxine, was 60 times greater in the B_6-deficient rats than in control animals on a complete diet.[5]

Both the desirable pharmacological effects and the toxicity of a drug may be related to the intact compound or to products derived from its metabolism. The acute toxicity of 2 possible metabolites of iproniazid, namely isonicotinic acid and IPH, was compared with that of the parent compound in studies in mice and rats. Whereas isonicotinic acid was relatively nontoxic in both of these species, IPH was 3 times as toxic as iproniazid in the rat and about twice as toxic in the mouse.

The rate of inactivation of a compound, its distribution in the body, and the nature and quantities of the metabolic products formed are likely to be specific for a given species or even peculiar to certain strains or individuals. In these studies iproniazid was found to exert specific hepatotoxic effects in mice of the AKR strain, but not in 3 other strains.

Experimental

Male albino rats (Holtzman strain) weighing from 50 to 75 gm. were used in the nutritional experiments; in the acute toxicity tests older rats of both sexes were used. The Swiss and C57/Bl mice were obtained from the Roswell Park Memorial Institute breeding colony, the DBA/2 and C3H mice from the Jackson Memorial Laboratories, Bar Harbor, Me., and the AKR mice from Millerton Research Farm, Millerton, N. Y. Except where indicated in the text, mature male mice weighing from 20 to 30 gm. were used. In all experiments the animals were grouped according to weight so that the average weights of individual groups in an experiment were comparable.

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* Hoffmann-La Roche Inc.

The complete purified ration contained 18 per cent casein, 73 per cent sucrose, 4 per cent each of salt mixture[6] and corn (Mazola) oil, 0.5 per cent cod-liver oil, and 0.2 per cent choline chloride, with the addition, for each 100 gm. of ration, of 1 mg. each of thiamine HCl, riboflavin, and pyridoxine HCl, 4 mg. of niacin, 6 mg. of calcium pantothenate, 15 mg. of inositol, 20 mg. of *p*-aminobenzoic acid, 10 mg. of α-tocopherol acetate, and 20 μg. each of biotin and folic acid.

The deficient diets were prepared by omitting the respective vitamins from the complete ration. Two niacin-deficient diets were used: No. 1 was the complete ration described above, modified to contain 9 per cent casein and 82 per cent sucrose; and diet No. 2 contained 9 per cent casein, 6 per cent gelatin, and 76 per cent sucrose, but identical in other respects to the complete ration. The diets and water were fed ad libitum.

A fluorometric method described by Lowe *et al.* was used for determining glutamic decarboxylase (GDC) activity.[7] Glutamic-oxaloacetic transaminase (GOT) activity and glutamic-pyruvic transaminase (GPT) activity were measured by the procedures of Lowry and his coworkers.[8, 9] Protein was determined in tissues by a modification of the Folin procedure.[10]

Results

The addition of iproniazid either to a complete diet or to diets deficient in pyridoxine, pantothenic acid, or niacin, resulted in pronounced toxic effects only in rats maintained on the diet that lacked vitamin B_6. The results shown in FIGURE 1 indicate the effect of different dietary levels of iproniazid on the growth of rats maintained on diets deficient in pyridoxine, pantothenic acid, or niacin. The experimental diets containing 0.005, 0.025, and 0.05 per cent iproniazid were supplied to 75-gm. rats, approximately 7.0, 35, and 70 mg. of the drug, respectively, per kilogram of body weight per day. The niacin deficiency was produced by the 9 per cent casein diet. Young rats were used in these experiments, which were of 5 weeks' duration.

Iproniazid depressed growth in the rat, but did not result in other symptoms of toxicity when added at the 0.025 and 0.05 per cent levels to a complete synthetic diet (FIGURE 1). Growth was not retarded in rats maintained on the niacin-deficient ration containing iproniazid, but hyperirritability and lesions of the tail and ears were evident, these lesions being much less severe than those observed in the pyridoxine-deficient animals. There was no evidence of a relationship between pantothenic acid deficiency and the toxicity of iproniazid; growth was not impaired, and the course of the deficiency was not altered.

At levels of 0.05 and 0.075 per cent in a pyridoxine-deficient diet, iproniazid suppressed growth in comparison to the weight response of animals on the deficient diet only (FIGURE 1). In contrast, there was a slight but significant increase in growth in the rats on this diet supplemented with 0.005 per cent iproniazid. Similar results were obtained in two other experiments.

In addition to the growth depression observed in the first group, necrotic lesions on the ears and tail and edema of the upper lip were evident during the third to fifth week of the experiment. During the fifth week the lesions began to heal and the edema subsided. Improvement continued until the experiment was terminated at the end of the sixth week. This reversal of effects may be associated with the anemia produced in rats treated with iproniazid, later followed by an increase in hemoglobin concentration even though the animals continued to receive the drug (L. O. Randall, unpublished observations).

In both the animals on the pyridoxine-deficient diet only and those on the diet plus iproniazid there was a loss of hair; this condition was more severe with animals on the high iproniazid intake and became progressively worse as the animals were continued on the diet.

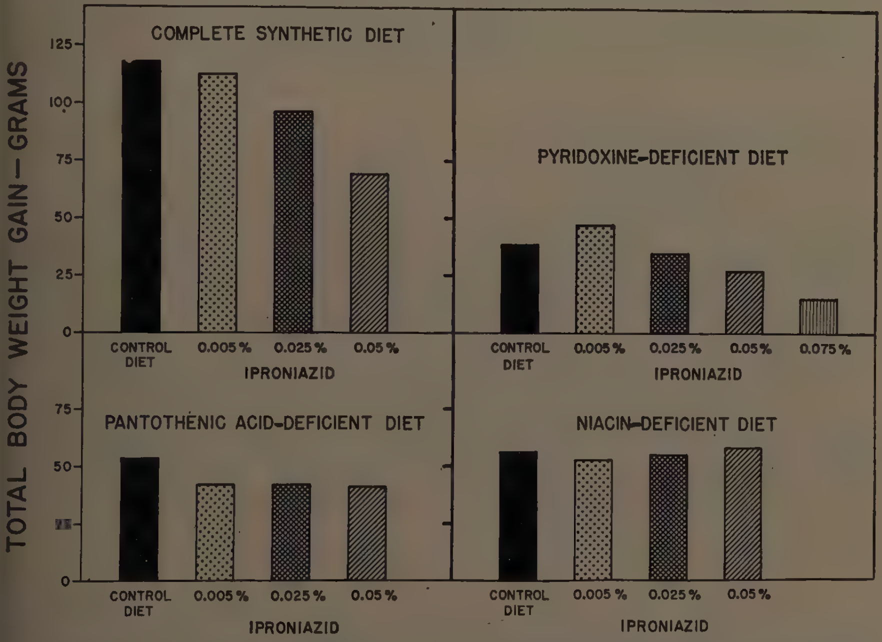

Figure 1. The effect of iproniazid on the growth of rats fed a complete diet and diets deficient in pyridoxine, pantothenic acid, or niacin. The average total weight gain for a period of 5 weeks is shown. There were 5 rats in each group.

The acute toxicity of iproniazid was determined in adult male rats previously maintained for 28 days on diets deficient in pyridoxine, pantothenic acid, or niacin. Impairment of growth was the single criterion used to indicate the vitamin-deficiency status of these animals. The niacin-deficient diet containing 9 per cent casein and 6 per cent gelatin was used in this study. Table 1 indicates that the acute toxicity (24-hour period of observation) of iproniazid in these vitamin-deficient animals is close to that observed in normal adult rats (LD_{50}, 168 mg./kg.).

Although it is common practice to use different species for studying the toxicity of a drug, different strains of a single species might also reveal important facts regarding the toxicity of a compound. Swiss mice and 3 inbred strains of mice, namely, DBA/2, AKR, and C57/Bl, were used to evaluate the chronic toxicity of iproniazid. Three diets were used: complete synthetic, pyridoxine-, and pantothenic acid-deficient. In each experiment 20 mice were separated into 4 groups. Group 1 was maintained on the complete or deficient diets, and the remaining 3 groups on these diets supplemented with iproniazid (5, 25, or 50 mg. per cent), designed to supply approximately 10, 50, and 100 mg. of the drug, respectively, per kg. of body weight per day.

TABLE 1
ACUTE TOXICITY OF IPRONIAZID IN RATS PREVIOUSLY DEPLETED OF PYRIDOXINE, PANTOTHENIC ACID, OR NIACIN

Diet	Iproniazid*	No. rats	Mortality
	mg./kg.		
Complete purified	100	5	1/5
	200	5	5/5
Pyridoxine-deficient	100	5	0/5
	200	5	5/5
Pantothenic acid-deficient	100	3	0/3
	200	5	4/5
Niacin-deficient	100	5	0/5
	200	5	5/5

* Iproniazid was administered intraperitoneally.

TABLE 2
LIVER INJURY AND MORTALITY OF AKR MICE ON COMPLETE SYNTHETIC DIET CONTAINING IPRONIAZID

Experiment*	Iproniazid	Liver injury†	Mortality
	Percentages in diet		
1	—	0/5	1/5
	0.005	0/5	2/5
	0.025	3/5	4/5
	0.050	2/5	3/5
2	—	0/10	3/10
	0.005	2/5	2/5
	0.025	0/5	0/5
	0.050	2/5	2/5
3	—	1/12	3/12
	0.025	7/12	8/12

* In Experiments 1 and 2 the observation period was 2 months; in Experiment 3, 1 month.
† Based on the gross observation of hemorrhagic spots in the liver.

No signs of iproniazid toxicity were observed in the Swiss, C57/Bl, or DBA/2 mice on either complete or deficient diets. In contrast, severe hepatic injury and a high incidence of mortality were observed in AKR mice receiving the complete diet (TABLE 2) or the vitamin-deficient diets supplemented with iproniazid. The liver injury was characterized grossly by hemorrhagic spots (FIGURE 2). This toxicity was not observed in all of the AKR mice treated with this drug, and it occurred with equal frequency in animals receiving 5, 25, and 50 mg. per cent of iproniazid in the diet. Microscopically, there were areas of necrosis in the livers of the AKR mice. These varied from small foci involving a few cells to large areas of infarctlike necrosis. A few polymorphonuclear leukocytes, lymphocytes, and macrophages were visible in these areas. Massive recent hemorrhage was found in the larger areas of necrosis at the time of autopsy. The pathology of the livers of AKR mice treated with iproniazid is being studied by L. Simpson of the Department of Pathology of Roswell Park Memorial Institute.

Consideration was given to the possibility that the toxicity of iproniazid in AKR mice might be due to an endemic infection. Three samples of liver from both control (complete diet) and treated mice (complete diet + 0.025 per cent iproniazid) were examined for a broad spectrum of bacteria. The livers were removed from animals sacrificed 1 week after they were placed on their respective diets. The cultures made from the liver of 1 control and 2 iproniazid-treated animals were sterile. None of the livers gave a positive test for *Salmonella* organisms. *Staphylococcus* was found in the liver of 1 control and 1 treated animal, and *Streptococcus* (enterococcus) was present in the liver of the other control animal. The occurrence of these organisms in laboratory mice is not unusual. The livers from the iproniazid-treated mice did not show the characteristic liver damage.

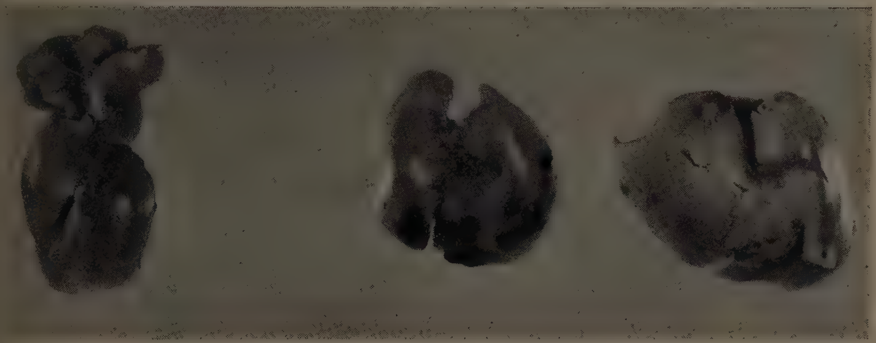

FIGURE 2. Liver injury in AKR mice given iproniazid in the diet. *Left*: liver from rat on complete synthetic diet; *center* and *right*, livers from rats on complete synthetic diet plus 0.025 per cent iproniazid.

While these results indicate that some of the AKR mice used carry potentially infectious organisms, this strain shows no gross evidence of illness or infection. These observations were made recently, and additional studies are required, particularly in mice with livers that show hepatic damage, before infection is ruled out as a relevant factor in the toxicity of iproniazid in AKR mice.

The acute lethal dose of iproniazid injected intraperitoneally was compared with that of its 2 possible metabolic end products,[11] IPH and isonicotinic acid, in several strains of mice and in normal and pyridoxine-deficient rats (TABLE 3). IPH was about 3 times as toxic as iproniazid in normal rats; isonicotinic acid was not lethal in either mice or rats at the highest amount injected (1750 mg./kg.). The LD_{50} for iproniazid was about the same in normal and pyridoxine-deficient rats, whereas INH was 3 times more toxic in rats deficient in pyridoxine than in normal animals.

Initially, it was observed that the acute toxicity of iproniazid in the normal adult rat was twice as high as that in immature pyridoxine-deficient rats. Further investigation revealed that the acute toxicity of iproniazid in the rat varies directly with the size of the animal used. The comparative LD_{50} values for iproniazid, administered by 3 different routes in rats of different age and weight, are shown in TABLE 4.

TABLE 3

MEDIAN LETHAL DOSE (LD_{50}) OF IPRONIAZID AND RELATED COMPOUNDS IN VARIOUS STRAINS OF MICE AND IN NORMAL AND PYRIDOXINE-DEFICIENT RATS

Compound	Species, strain	LD_{50}, mg./kg.*
Iproniazid	Mouse, DBA/2	500
	Mouse, C57Bl†	438
	Mouse, AKR	520
	Mouse, C_3H	792
	Mouse, Swiss	540
	Rat, immature‡	230, normal
	Rat, immature	225, B_6-def.
Isopropyl hydrazine	Mouse, Swiss†	320
	Rat, mature	58, normal
	Rat, mature	34, B_6-def.
Isonicotinic acid hydrazine	Mouse, Swiss†	150
	Rat, mature†	400, normal
	Rat, mature	142, B_6-def.
Isonicotinic acid	Mouse, Swiss†	>1750
	Rat, mature†	>1750

* The acute toxicity of a single dose administered intraperitoneally.

† Female animals.

‡ The average weight of the immature rats was 117 gm. and for the mature animals 212 gm.

TABLE 4

MEDIAN LETHAL DOSE (LD_{50}) OF IPRONIAZID ADMINISTERED BY VARIOUS ROUTES TO IMMATURE AND ADULT RATS

Rat studied	Route of administration	LD_{50}, mg./kg.*
Adult (300–400 gm.)	Oral	188
	Subcutaneous	168
	Intraperitoneal	168
Immature (140–170 gm.)	Oral	244
	Subcutaneous	275
	Intraperitoneal	230
Immature (50–63 gm.)	Oral	>375
	Subcutaneous	>375
	Intraperitoneal	>375

* Based on 24 hours of observation. Five animals per dose level and at least 4 levels for each compound. The LD_{50} values were calculated by the method of Litchfield and Wilcoxon.[12]

In other studies, iproniazid, INH, and IPH were tested *in vivo* for their effects on the activity of 3 enzymes that require pyridoxal phosphate as a cofactor. None of these compounds significantly altered the activities of the transaminase enzymes in brain or liver (TABLE 5). There was a marked decrease, however, in the glutamic decarboxylase activity of rat brain after treatment for 1 week with INH or IPH; in contrast, iproniazid had no effect on the activity of this enzyme (TABLE 6).

TABLE 5

EFFECT OF IPRONIAZID, ISONIAZID, AND ISOPROPYL HYDRAZINE ON THE GLUTAMIC-PYRUVIC, AND GLUTAMIC-OXALOACETIC TRANSAMINASE ACTIVITIES IN RAT BRAIN AND LIVER

Treatment†	Enzyme activity* (average values for 5 rats)			
	GPT		GOT	
	Brain	Liver	Brain	Liver
B_6-deficient diet (1)	1.35 ± 0.1‡	4.71 ± 0.36	71.7 ± 6.7	119 ± 12
Diet 1 + 0.075% iproniazid	1.51 ± 0.1	5.93 ± 1.3	72.9 ± 4.5	100 ± 2.5
Diet 1 + 0.10% isoniazid	1.59 ± 0.04	5.51 ± 0.89	69.7 ± 2.7	102 ± 6.7
Diet 1 + 0.02% IPH	1.38 ± 0.2	7.40 ± 1.1	78.5 ± 13	112 ± 8.0

* The period of treatment was 7 days. The rats were placed on the synthetic diet or the diet plus drug on day 1.

† mMoles of substrate utilized per gram of protein per hour at 37° C.

‡ Standard deviation.

TABLE 6

EFFECT OF IPRONIAZID, ISONIAZID, AND ISOPROPYL HYDRAZINE ON THE GLUTAMIC DECARBOXYLASE ACTIVITY IN RAT BRAIN

Treatment*	Glutamic decarboxylase			
	Endgenous		Pyridoxal phosphate added	
	Activity†	Per cent control	Activity	Per cent control
Complete diet (1)	0.081 ± 0.003‡	—	0.235 ± 0.03	—
Diet 1 + 0.075% iproniazid	0.077 ± 0.01	92	0.218 ± 0.01	93
Diet 1 + 0.10% INH	0.069 ± 0.01	82	0.201 ± 0.02	86
Diet 1 + 0.02% IPH	0.074 ± 0.01	88	0.208 ± 0.01	89
B_6-deficient diet (2)	0.067 ± 0.01	—	0.196 ± 0.01	—
Diet 2 + 0.075% iproniazid	0.054 ± 0.01	81	0.197 ± 0.02	100
Diet 2 + 0.10% isoniazid	0.023 ± 0.004	34	0.176 ± 0.01	90
Diet 2 + 0.02% IPH	0.036 ± 0.01	54	0.191 ± 0.02	98

* The period of treatment was 7 days. The rats were placed on the synthetic diet or the diet plus drug on day 1.

† Millimoles of substrate utilized per gram of brain protein per hour at 37° C.

‡ Standard deviation.

Discussion

Clear-cut evidence of toxicity was obtained in rats maintained on diets deficient in pyridoxine and containing 50 and 75 mg. per cent of iproniazid. With the exception of retardation in growth, the other manifestations of toxicity were different from those usually observed in rats deprived of pyridoxine or treated with its antimetabolites. Thus, 2 antagonists of pyridoxine, namely, desoxypyridoxine and INH, exert highly specific and much more severe toxic effects than iproniazid when administered to rats on diets lacking the vitamin.[1] In recent studies, Wiss *et al.* (unpublished observations) reported a slight increase in the excretion of pyridoxine in the urine of rats treated with iproniazid, whereas Marusich and De Ritter (unpublished observations), failed to observe this effect. These conflicting data and our observations indicate the need for further studies before any significance is attached to a relationship between iproniazid toxicity and pyridoxine metabolism.

Additional information on this point was sought in studies comparing the toxicity of iproniazid with INH and IPH in normal and pyridoxine-deficient rats. The toxicity of INH is characterized by severe convulsions and is much greater in pyridoxine-deficient rats. Although the acute LD_{50} for iproniazid was higher than that found for IPH, both compounds were equally toxic in normal and B_6-deficient animals, and only at doses close to their LD_{90} did they induce convulsions. Thus, unlike that of INH, the toxicity of iproniazid is not increased in rats previously depleted of pyridoxine.

When added to a complete diet or diets deficient in pantothenic acid or niacin, iproniazid did not depress the growth of rats, and only in the niacin-depleted rats were moderate symptoms of toxicity noted. The level of iproniazid determined its effect in a pyridoxine-deficient diet. In comparison to rats on the B_6-deficient diet only, a slight but reproducible increase in weight occurred in animals maintained on the diet supplemented with 0.005 per cent iproniazid. We have not studied further the weight gain that occurs under these conditions. It may be that, similar to its reported action in man, iproniazid results in a favorable weight response by stimulating the appetite and thereby increasing the amount of food taken. Another possibility is that it acts in a manner similar to that postulated for Ω-methyl-pyridoxine, an antimetabolite of vitamin B_6: initially, this compound stimulates the growth of rats. One interpretation proposed was that the antagonist could displace pyridoxal phosphate from certain enzymes to which it is bound, resulting in a temporary redistribution of the available cofactor.[13]

It was thought that iproniazid or one of its metabolites might specifically inhibit certain enzymes that require pyridoxal phosphate as a cofactor. The glutamic decarboxylase activity of rat brain was markedly inhibited *in vivo* by INH and IPH added to diets deficient in pyridoxine, whereas iproniazid tested similarly did not inhibit this enzyme to any significant extent. As part of the same study, the activities of two transaminase enzymes (glutamic-oxaloacetic transaminase and glutamic-pyruvic transaminase) were measured in brain and liver. No effect of the drug was observed.

Clinically, it appears that liver injury is the most important toxic effect produced by iproniazid. In the present studies hepatic damage and a high incidence of mortality were observed in AKR mice maintained on diets supplemented with iproniazid. This effect was noted in mice on the complete diet, as well as in those on the pyridoxine- and pantothenic acid-deficient diets, and seemed to be independent of the level of iproniazid in the rations. In similar studies carried out in DBA/2, C57/Bl, and Swiss mice, there was no evidence of liver pathology, and the animals survived for a period of 2 months. At present we have no explanation for the specific toxicity of iproniazid in AKR mice. Currently we are investigating the possibility that this result may be due to an infection endemic in these mice that is potentiated by iproniazid. It should be noted, however, that the AKR mice used in these studies appeared to be normal, healthy animals.

The potential usefulness of determining the toxicity of a compound in more than one strain of mice was demonstrated by the severe liver damage in AKR mice treated with iproniazid, that was not observed in the three other strains studied. Although the significance of the effects noted in rats maintained on pyridoxine-deficient diets supplemented with iproniazid is not clear, it would seem that this approach to studying relationships between essential nutrients and drugs is capable of yielding useful information. Similarly, comparative studies on the toxicity of a drug and its metabolites may indicate the specific components that are primarily responsible for its activity and toxicity.

Summary

The effects of iproniazid in rats maintained on complete diets and diets deficient in pyridoxine, pantothenic acid, or niacin were studied. The addition of 0.05 and 0.075 per cent iproniazid to a pyridoxine-deficient diet had a pronounced toxic effect, characterized by loss of hair, edema, hemorrhagic lesions of the ears and tail, and inhibition of growth. Severe liver damage and a high incidence of mortality were observed in AKR mice fed complete, pantothenic acid-, or pyridoxine-deficient diets containing iproniazid. Under similar conditions iproniazid was not toxic in DBA/2, C57/Bl, or Swiss mice. The LD_{50} for iproniazid was about the same in normal and pyridoxine-deficient rats, whereas isonicotinic acid hydrazide was 3 times more toxic in rats deficient in pyridoxine than in normal animals. Isopropylhydrazine was 3 times as toxic as iproniazid in the normal rat, and isonicotinic acid was not lethal in mice or rats at the highest amount injected, namely, 1750 mg./kg. Glutamic-decarboxylase activity in the brain of pyridoxine-depleted rats was markedly inhibited following treatment with isonicotinic acid hydrazide or isopropylhydrazine for 1 week; iproniazid did not produce a significant decrease in the activity of this enzyme. None of these compounds significantly altered the activities of glutamic-oxalacetic transaminase or glutamic-pyruvic transaminase in brain or liver.

Acknowledgments

I thank Charles A. Nichol for many helpful discussions and for his assistance in the preparation of this paper. The capable technical assistance of Albert Clark, Richard Milholland, William Kremer, and Delores Klein is gratefully acknowledged.

References

1. Rosen, F. 1955. Effect of isonicotinic acid hydrazide on niacin and pyridoxine metabolism in rats. Proc. Soc. Exptl. Biol. Med. **88:** 243–246.
2. Ungar, J., K. R. Parkin, E. G. Tomich & P. W. Muggleton. 1954. Effect of pyridoxine on the action of isoniazid. Lancet. **2:** 220–221.
3. Yoneda, M. & N. A. Asano. 1953. Competitive action of isonicotinic acid hydrazide and pyridoxal in the amino acid decarboxylation of *Escherichia coli*. Science. **117:** 277–279.
4. Yoneda, M., N. Kato & M. Okajuna. 1952. Competitive action of isonicotinic acid hydrazide and vitamin B_6 in the formation of indole by *E. coli*. Nature. **170:** 803.
5. Rosen, F., J. F. Holland & C. A. Nichol. 1957. Effects of 2-methyl- and 2-methyl-

mercapto-4-amino-5-hydroxymethyl pyrimidine in thiamin- or pyridoxine-depleted rats. Proc. Am. Assoc. Cancer Research. **2:** 243.
6. HUBBEL, R. G., L. B. MENDEL & A. J. WAKEMAN. 1937. A new salt mixture for use in experimental diets. J. Nutrition. **14:** 273–281.
7. LOWE, I. P., E. ROBINS & G. A. SCHMALZ. 1957. A fluorometric method for the microdetermination of glutamic decarboxylase. Federation Proc. **16:** 214.
8. LOWRY, O. H., N. R. ROBERTS & M.-L. W. CHANG. 1956. The analysis of single cells. J. Biol. Chem. **222:** 97–107.
9. ROSEN, F., N. R. ROBERTS & C. A. NICHOL. 1959. Glucocorticosteroids and transaminase activity. I. Glutamic-pyruvic transaminase in four physiological conditions associated with gluconeogenesis. J. Biol. Chem. **234:** 476–480.
10. LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR & R. J. RANDALL. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193:** 265–275.
11. DE RITTER, E., L. DREKTER, J. SCHEINER & S. H. RUBIN. 1952. Urinary excretion of hydrazine derivatives of isonicotinic acid in normal humans. Proc. Soc. Exptl. Biol. Med. **79:** 654–658.
12. LITCHFIELD, J. T. & F. WILCOXON. 1949. A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exptl. Therap. **96:** 99–113.
13. SANDMAN, R. & E. E. SNELL. 1955. Some effects of Ω-methyl analogues of vitamin B_6 in rats. Proc. Soc. Exptl. Biol. Med. **90:** 63–67.

Discussion of the Paper

DAVID BAIRD COURSIN (*Research Institute, St. Joseph's Hospital, Lancaster, Pa.*): I congratulate Rosen on his concise presentation of the excellent experimental work that his group has done on the effects of hydrazides in vitamin-deficient animals. His data indicate that the addition of increasing dosages of iproniazid (Marsilid) may alter the clinical course and enzyme activity of these animals. This was particularly evident in the pyridoxine-deficient AKR strain of mice, which showed peripherovascular changes, increased nervous system excitability, and hemorrhagic phenomena in the liver. While some of these findings are not quite the same as in the human, they serve as important guides for the future experimental work planned by Rosen.

TABLE 1

EFFECT OF RIBOFLAVIN AND PYRIDOXINE DEFICIENCIES ON THE MONOAMINE OXIDASE ACTIVITY OF RAT LIVER*

Activity Is Expressed as µl. O_2 Uptake/Hour/100 mg. Wet Wt. of Tissue. All Values Have Been Corrected for O_2 Uptake in the Absence of (Added) Substrate.[2]

Diet	Week					
	1	3	4	5	6	7
A: tyramine						
Complete	230	225	352	—	390	270
Deficient in vitamin B_6	340	234	352	340	354	375
Deficient in vitamin B_2	98	165	132	132	60	75
Deficient in both vitamins	254	210	228	128	155	105
B: isoamylamine						
Complete	94		132	—	90	90
Deficient in vitamin B_6	102		136	106	120	105
Deficient in vitamin B_2	70		76	63	30	30
Deficient in both vitamins	72		84	72	60	45

* Data of T. L. Sourkes, Allan Memorial Institute.

Another important aspect of vitamin interrelationships with monoamine oxidase has recently been studied by T. L. Sourkes of the Allan Memorial Institute of Psychiatry in Montreal, Que., Canada. Sourkes has kindly permitted me to use some of his group's new data[1] on rats maintained on selected dietary regimens. These diets were (1) complete, (2) vitamin B_6-deficient, (3) vitamin B_2-deficient, and (4) deficient in both of these vitamins.[2] Members of each group were sacrificed at weekly intervals and their liver

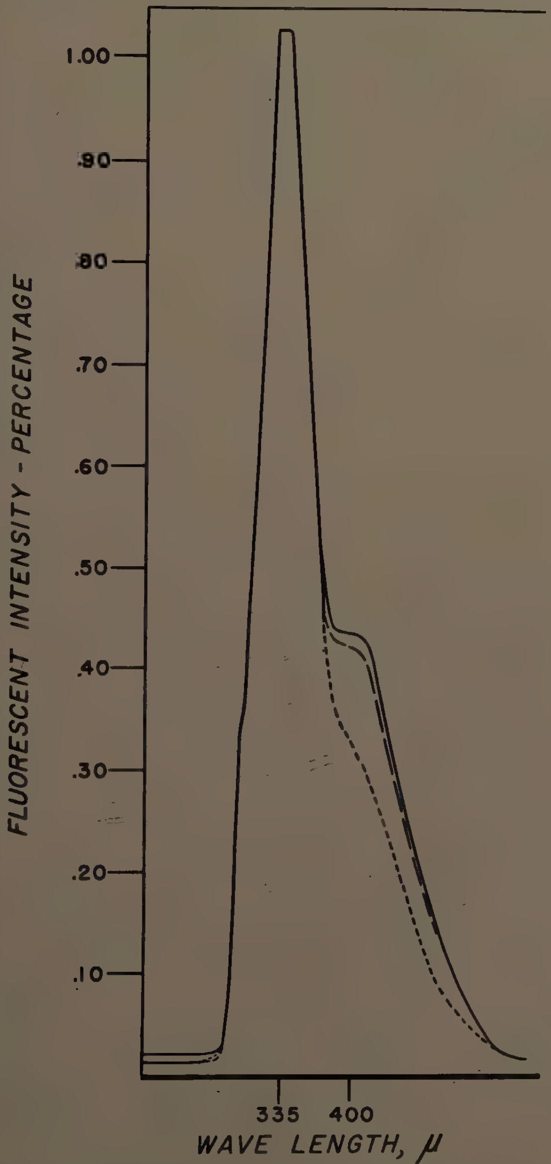

Figure 1. Whole blood sample taken during iproniazid toxicity. *Solid line* shows total fluorescent transmission; *broken line* shows fluorescence of pyridoxamine remaining after the destruction of pyridoxal with 30 per cent hydrogen peroxide; *dotted line* shows the blank produced by irradiation with ultraviolet light to destroy the pyridoxamine.

homogenates assayed for monoamine oxidase content. The technique was a manometric one utilizing tyramine and isoamylamine as substrates with measurement of microliters of oxygen uptake per hour per 100 mg. of wet tissue.[1]

TABLE 1 lists the data for a 7-week period of study. The controls are noted

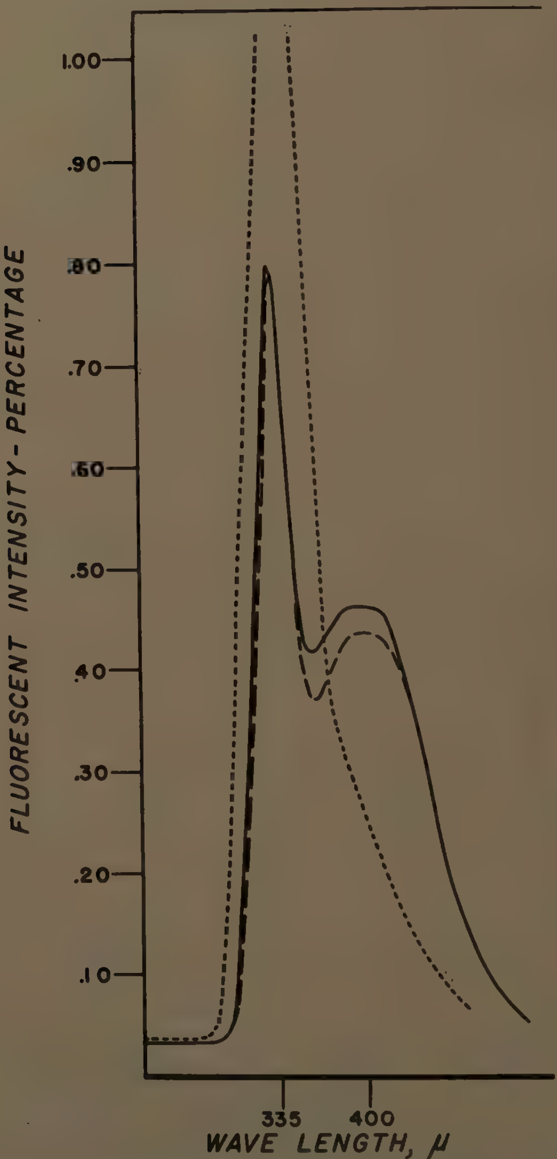

FIGURE 2. Whole blood sample 7 days after the withdrawal of iproniazid. *Solid line* shows increased content of vitamin B_6; *broken line* shows increased pyridoxamine following the destruction of the larger quantity of pyridoxal; *dotted line* shows the residual blank after irradiation with ultraviolet light to destroy the pyridoxamine.

to have rather stable values varying somewhat with increasing age. The pyridoxine-deficient animals were within the normal range or higher. The riboflavin-deficient rats exhibited a marked reduction (from one third to one half, compared with controls), and it is of interest to note that the depression of activity was evident within the first week of the experiments. Finally, the data indicate a definite sparing of the monoamine oxidase activity as a result of joint pyridoxine and riboflavin deficiency.

In considering these problems in the human, some of the material we obtained during a recent study of apparent iproniazid toxicity is of interest. The patient was a 65-year-old female with mental depression who had been treated with iproniazid from June 1958. She initially received 25 mg. 3 times daily and subsequently was maintained on 25 mg. twice daily. She was admitted to the hospital in September 1958 with a history of progressive decrease in appetite, weight loss, nausea, vomiting, and acute deepening jaundice. Extensive diagnostic studies were undertaken, of which the liver function tests were the most important. These showed elevated alkaline phosphatase, prolonged prothrombin time, positive cephalin flocculation, positive thymol turbidity, and Van den Bergh direct, immediate, and indirect, 28 mg. per cent. Blood-ammonia level was 1.75 μg./cc.; serum glutamic-oxaloacetic transaminase was 250 units.

The iproniazid medication was discontinued and the patient carried on supportive therapy. Her jaundice has now virtually disappeared, and the abnormal laboratory findings have returned to normal limits.

Throughout the patient's hospital stay the vitamin B_6 content of her whole blood was repeatedly measured by the spectrophotofluorometric method recently described by our laboratory.[3] FIGURE 1 shows the small quantity of pyridoxal and pyridoxamine present at the time of admission during the patient's toxic phase. FIGURE 2 demonstrates the increase in these components 7 days after stopping iproniazid therapy and before supplemental pyridoxine was given. This progressive rise was further increased with added pyridoxine and has now stabilized.

The importance of riboflavin and pyridoxine to the mechanism of iproniazid toxicity is not yet clear. A direct deficiency-producing effect, such as that seen with isonicotinic acid hydrazide and pyridoxine, remains to be demonstrated. However, it is quite possible that the prolonged administration of iproniazid may produce a chronic subclinical deficiency of vitamin B_6 in humans that could contribute to iproniazid toxicity. Certainly, by the time hepatocellular changes have occurred, there are marked alterations in enzyme activity and depletion of vitamin stores.

Acknowledgment

I thank H. Glah and P. Hemler for their cooperation in supplying information on their patient.

References

1. Lagnado, J. R. & T. L. Sourkes. 1958. Can. J. Biochem. Physiol. **36:** 587.
2. Sourkes, T. L. 1958. Rev. Can. Biol. In press.
3. Coursin, D. B. & V. C. Brown. 1958. Proc. Soc. Exptl. Biol. Med. **98:** 315–318.

SERIAL HEPATIC STUDY IN PATIENTS TREATED WITH IPRONIAZID

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As stated by Davis,[1] along with the encouraging therapeutic results of the use of iproniazid (Marsilid*) came reports of undesirable effects. Some of these are due to overdosage; others consist of potentiation or prolongation of the action of other drugs. The third type of undesirable reaction probably due to Marsilid is a hepatocellular type of liver damage that is impossible to distinguish from viral hepatitis.

The purpose of the present investigation was to evaluate the possible toxic effects of Marsilid on the human liver. We studied this problem by a series of clinical, biochemical, and biopsy examinations of patients receiving Marsilid.

Review of the Literature

In their preliminary report on the treatment of bone and joint tuberculosis with Marsilid, Bosworth and his associates[2] described one case of jaundice which, although severe, recovered spontaneously. In their later publications[3, 4] this group of investigators described a total of 4 cases of hepatitis with jaundice among 142 patients treated with Marsilid. Nevertheless, these authors could not be certain that these cases were really due to an hepatoxic effect of the drug.

In 3 or 5 patients who developed nervous symptoms during Marsilid treatment, Scherbel[5] observed temporary abnormal bromsulphalein excretion.

De Verteuil and Lehmann[6] treated 6 psychotic patients with Marsilid. In one of them a fatal necrosis of the liver occurred on a low dosage schedule.

Settel[7] reported one case of jaundice among 48 depressed patients treated with Marsilid. No details of the case are given.

Zetzel and Kaplan[8] reported 3 cases of jaundice appearing in women who were receiving Marsilid in relatively small doses. The liver disturbances noted were far from mild, one resulting in death and one in apparent recovery after many weeks of hospitalization; another patient remained seriously ill, with evidence of severe disruption of liver function at the time of writing. The authors state that there is no unequivocal evidence that relates Marsilid etiologically to the observed liver disease, but that the possibility exists that it represents the toxic effect of the drug.

Walker (cited by Frantz[9]) has observed diffuse necrosis of the liver in one patient who became jaundiced and died shortly after cessation of two months of therapy with Marsilid. Frantz[9] reports a case of acute necrosis of the liver in association with Marsilid therapy of about 2½ months' duration. The latter investigator does not claim that iproniazid was definitely the toxic agent, since the illness could have represented a transmitted inoculation hepatitis. In this case the laboratory findings were those of hepatocellular jaundice.

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* Hoffmann-La Roche Inc.

According to the data collected by Frey and Herrero for Hoffmann-La Roche & Co., Ltd., Basle, Switzerland, (personal communication) the total number of cases treated with Marsilid in the United States is about 500,000. In this group 102 cases of hepatitis have been reported, 21 of which have been fatal. These figures correlate well with those given by Popper (personal communication to H.D.).

Material and Methods

Two groups of patients were studied. The first consisted of 48 psychiatric patients ranging in age from 20 to 68 years who received Marsilid for depression of different etiologies (organic and neurotic). The second group comprised 21 patients with status anginosus; members of this group ranged in age from 39 to 76 years. All these patients received Marsilid daily, the initial dose being from 100 to 150 mg. and the maintenance dose from 25 to 100 mg. per day.

TABLE 1

LABORATORY TESTS USED*

Test	Considered abnormal
Prompt direct serum bilirubin[10]	Over 0.25 mg./100 ml.
Total serum bilirubin[10]	Over 1 mg./100 ml.
Serum alkaline phosphatase[10]	Over 6 units
Thymol turbidity[10]	Over 8 units
Thymol flocculation[10]	+ or more
Cephalin cholesterol flocculation[10]	++ and +++
Colloidal red[10]	++ and +++
Zinc turbidity[11]	Over 16 units
Prothrombin time[12]	Less than 80 per cent of normal control
Labile factor[13]	Less than 80 per cent of normal control
Serum iron[13]	Over 150 μg./100 ml.
Serum glutamic oxaloacetic transaminase[14]	Over 55 units/ml.
Serum glutamic-pyruvic transaminase[14]	Over 50 units/ml.
Bromsulphalein retention (30 min., 5 mg./kg.)[10]	Over 10 per cent
Serum albumin[10]	Less than 3 gm./100 ml.
Serum gamma globulin[10]	Over 2 gm./100 ml.

* For 15 patients in the psychiatric group a total of 25 liver biopsies was performed.

The psychiatric patients* were hospitalized during the observation period and did not receive any other drug that could be hepatotoxic. Most of the cardiac patients were ambulatory. The maintenance period of Marsilid therapy ranged from 30 to 150 days. The average observation period was 90 days for the psychiatric patients and 65 days for the cardiac cases. From both groups 44 patients were observed for 70 days or more.

All of the patients were carefully controlled both from the clinical and

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* The psychiatric patients were under the care of Agustin Tellez, Miguel Estay, and Juan Brzovic at the Psychiatric Hospital, Santiago, Chile.

TABLE 2
SERIAL HEPATIC TESTS IN 48 PSYCHIATRIC PATIENTS TREATED WITH MARSILID

Duration of therapy (days)		Test							
		Prompt direct bilirubin (mg./100 ml.)	Total bilirubin (mg./100 ml.)	Alkaline phosphatase (units)	Oxaloacetic transaminase (units/ml.)	Pyruvic transaminase (units/ml.)	Bromsulphalein (per cent retention)	Prothrombin complex (per cent of normal)	Labile factor (per cent of normal)
Control	No. of obser.	48	48	48	48	48	44	48	48
	Av. ± S.D.	0.10 ± 0.06	0.31 ± 0.17	4 ± 1.6	38 ± 6.4	30 ± 7.3	5.1 ± 1.8	93 ± 10	93 ± 10
	No. abnormals	0	0	5	1	0	0	3	3
	Per cent abnormals	0	0	10.4	2.1	0	0	6.2	6.2
10	No. of obser.	46	46	46	46	46		46	46
	Av. ± S.D.	0.11 ± 0.05	0.33 ± 0.16	3.9 ± 1.4	34 ± 7.9	31 ± 8.3		93 ± 10	93 ± 10
	No. abnormals	0	0	5	0	1		5	5
	Per cent abnormals	0	0	11	0	2.2		10.8	10.8
20	No. of obser.	47	47	47	47	47		47	47
	Av. ± S.D.	0.10 ± 0.05	0.35 ± 0.29	3.7 ± 2.3	38 ± 6.5	30.6 ± 7.1		94 ± 10	94 ± 10
	No. abnormals	0	2	3	1	0		3	3
	Per cent abnormals	0	4.2	6.4	2.1	0		6.4	6.4
30	No. of obser.	44	44	44	44	44	37	44	44
	Av. ± S.D.	0.09 ± 0.05	0.28 ± 0.27	0.8 ± 1.3	38.6 ± 6	29.3 ± 7	5.4 ± 1.1	94 ± 11	94 ± 11
	No. abnormals	1	1	2	1	0	0	4	4
	Per cent abnormals	2.3	2.3	4.6	2.3	0	0	9.2	9.2
50	No. of obser.	41	41	41	41	41		41	41
	Av. ± S.D.	0.10 ± 0.05	0.34 ± 0.22	4.4 ± 2.6	38 ± 6	28.5 ± 7		98 ± 6	98 ± 6
	No. abnormals	0	1	4	0	0		1	1
	Per cent abnormals	0	2.4	9.7	0	0		2.4	2.4
70	No. of obser.	36	36	36	36	36		36	36
	Av. ± S.D.	0.12 ± 0.06	0.31 ± 0.13	3.8 ± 1.4	36 ± 6.7	29 ± 6.7		99 ± 2	99 ± 2
	No. abnormals	0	0	2	0	0		0	0
	Per cent abnormals	0	0	6.1	0	0		0	0

90	No. of obser.	35	35	35	35	35	32	35	35
	Av. ± S.D.	0.18 ± 0.5	0.36 ± 0.8	4.3 ± 1.7	44 ± 43	42 ± 87	5.8 ± 7.2	98 ± 4	98 ± 4
	No. abnormals	1	1	3	1	1	1	0	0
	Per cent abnormals	2.9	2.9	8.6	2.9	2.9	3.1	0	0
110	No. of obser.	26	26	26	26	26		26	26
	Av. ± S.D.	0.11 ± 0.06	0.28 ± 0.13	4.5 ± 1.2	35 ± 4.6	27 ± 4.6		99 ± 3	99 ± 3
	No. abnormals	1	0	3	0	0		0	0
	Per cent abnormals	3.8	0	11.5	0	0		0	0
130	No. of obser.	18	18	18	18	18		18	18
	Av. ± S.D.	0.08 ± 0.03	0.23 ± 0.24	4.7 ± 0.79	36 ± 4	27 ± 4		99 ± 1	99 ± 1
	No. abnormals	0	0	2	0	0		0	0
	Per cent abnormals	0	0	11	0	0		0	0

TABLE 3

SERIAL HEPATIC TESTS IN 48 PSYCHIATRIC PATIENTS TREATED WITH MARSILID

Duration of therapy (days)		Test							
		Thymol turbidity (units)	Thymol flocculation	Cephalin flocculation	Colloidal red	Zinc turbidity (units)	Serum albumin (gr./100 ml.)	Gamma globulin (gr./100 ml.)	Serum iron (μg./100 ml.)
Control	No. of obser.	48	48	48	48	48	48	48	48
	Av. ± S.D.	3.9 ± 1.9	—	—	—	12 ± 4.3	3.8 ± 0.51	1.49 ± 0.36	113 ± 30
	No. abnormals	7	7	9	2	7	4	2	5
	Per cent abnormals	14.6	14.6	18.7	4.2	14.6	8.4	4.2	10.4
10	No. of obser.	46	46	46	46	46			46
	Av. ± S.D.	3.8 ± 2	—	—	—	12.4 ± 4.4			112 ± 23
	No. abnormals	9	8	6	2	9			2
	Per cent abnormals	19.5	17.3	13	4.3	19.5			4.3
20	No. of obser.	47	47	47	47	47			47
	Av. ± S.D.	3.5 ± 1.7	—	—	—	11.2 ± 4.2			113 ± 31
	No. abnormals	5	5	7	4	8			5
	Per cent abnormals	10.7	10.7	14.6	8.4	17.4			10.7
30	No. of obser.	44	44	44	44	44	44	44	44
	Av. ± S.D.	3.6 ± 1.7	—	—	—	10.8 ± 3.6	3.6 ± 0.43	1.47 ± 0.23	110 ± 25
	No. abnormals	2	2	10	4	4	4	0	1
	Per cent abnormals	4.6	4.6	22.7	9.2	9.2	9.2	0	2.3
50	No. of obser.	41	41	41	41	41			41
	Av. ± S.D.	3.6 ± 1.4	—	—	—	10.8 ± 4.2			112 ± 28
	No. abnormals	2	7	9	4	8			1
	Per cent abnormals	4.9	17	22	9.7	19.5			2.4
70	No. of obser.	36	36	36	36	36			36
	Av. ± S.D.	3.9 ± 2.1	—	—	—	10.4 ± 3.8			113 ± 29
	No. abnormals	7	6	7	3	1			1
	Per cent abnormals	19.4	16.7	19.4	8.3	3			3

90	No. of obser.	35	35	35	35	35	35	35	35
	Av. ± S.D.	4.1 ± 1.7	—	—	—	10.7 ± 3.2	3.6 ± 0.4	1.6 ± 0.28	118 ± 24
	No. abnormals	4	10	8	7	1	1	2	3
	Per cent abnormals	11.4	28.6	22.2	20	2.9	2.9	5.8	8.6
110	No. of obser.	26	26	26	26	26			26
	Av. ± S.D.	4 ± 1.6	—	—	—	12.4 ± 3			116 ± 19
	No. abnormals	4	8	5	6	2			1
	Per cent abnormals	15.4	30.8	19	23	7.6			3.8
130	No. of obser.	18	18	18	18	18			18
	Av. ± S.D.	3.5 ± 1.7	—	—	—	12 ± 4			116 ± 18
	No. abnormals	0	3	2	4	2			1
	Per cent abnormals	0	16.6	11	22	11			5.5

laboratory standpoints while receiving the drug and up to one month following the end of the treatment. The clinical and laboratory controls were performed before starting the drug and at regular intervals throughout the observation period. The tests used are listed in TABLE 1.

Results

The results of the serial laboratory tests in the psychiatric patients are summarized in TABLES 2 and 3. The left-hand columns indicate when the tests were performed, beginning with the initiation and ending with the 130th day after the beginning of Marsilid therapy (initial, 10, 20, 30, 50 70, 90, 110, and 130 days.) Under each test, the total number of patient examined, the average findings, their standard deviation, and the number an percentage of abnormal results obtained are shown. The initial values o this group of psychiatric patients indicate the usual proportion of abnorma results we obtain in apparently normal subjects.

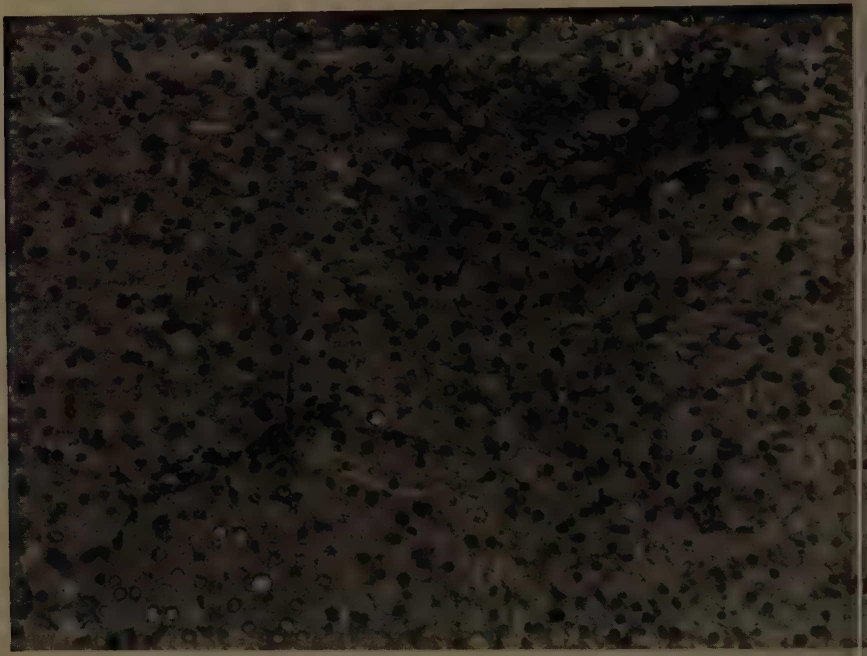

FIGURE 1. Serial histological studies in patient No. 42, who developed hepatitis. Befor initiation of treatment: normal liver architecture, very slight parenchymal alteration, an portal infiltration. Hemotoxylin and eosin stain. ×240.

It may be seen that until 70 days of treatment no abnormality was foun except for the serum bilirubin. In the examination performed 20 days afte beginning the treatment 2 patients (cases Nos. 2 and 36) showed an increas in the total serum bilirubin to 1.30 and 1.72 mg., respectively. In case N 2, treatment was continued and the serum bilirubin subsequently returne to normal; in case No. 36 treatment was discontinued. No other abnormality accompanied the elevation of serum bilirubin in these 2 patients; in the latter (case No. 36) a liver biopsy was performed. A third patient (case No. 9) showed an increase in prompt direct and total bilirubins to 0.36 and 1.86 mg., respectively, in the examination performed 30 days after inception of treatment. Therapy was discontinued, but was resumed after bilirubin returned to normal without reappearance of the abnormality through an additional observation period of 15 days.

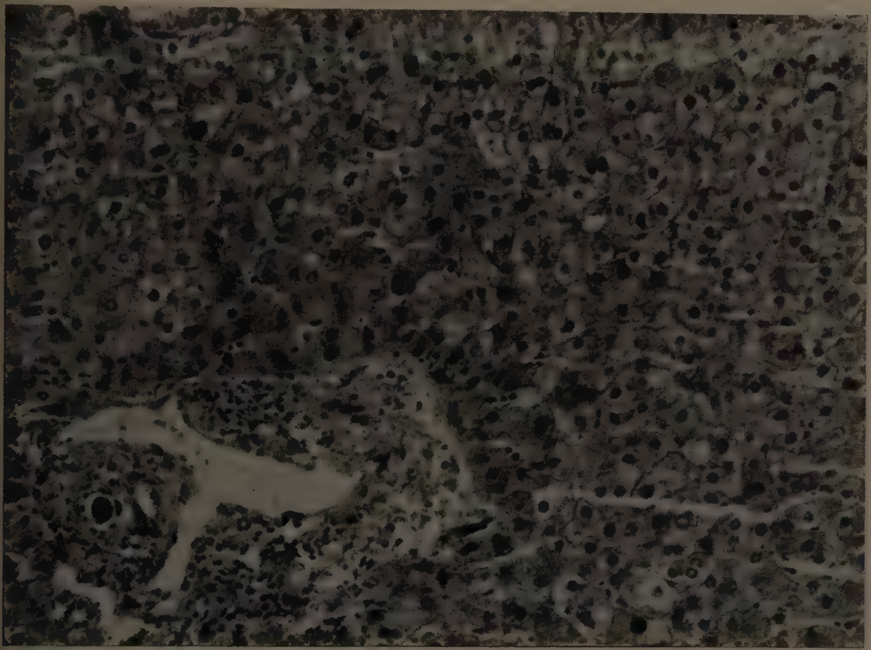

FIGURE 2. Serial histological studies in patient No. 42, who developed hepatitis. After 74 days of Marsilid therapy: less parenchymal alteration than in the preceding biopsy, very slight portal infiltration, and slight conjunctival proliferation. Hematoxylin and eosin stain. ×240.

In the laboratory control performed at 50 days, another patient (case No. 5) showed a mild elevation of total serum bilirubin to 1.1 mg./100 ml.; this was disregarded, and treatment continued, with subsequent return of bilirubin to normal.

In the control performed at 90 days after starting the treatment with Marsilid, one case of hepatitis was discovered (case No. 42). The history of this case, a male aged 56 years with known hypertension and with an anxiety syndrome apparently of involutional origin, is as follows. He received Marsilid (100 mg. daily) from May 5 to June 6, when the dose was increased to 150 mg. daily; on July 16 it was reduced to 50 mg. daily.

On the day when treatment was initiated, physical examination revealed a normal liver; laboratory tests and liver biopsy were performed. During the

TABLE 4

LABORATORY EXAMINATIONS IN PATIENT NUMBER 42 WHO DEVELOPED HEPATITIS

Test	Date					
	5/3/1958	6/4/1958	7/16/1958	8/5/1958	8/25/1958	9/30/1958
Prompt direct bilirubin (mg./100 ml.)	0.18	0.14	0.11	3.38	1.38	0.06
Total bilirubin (mg./100 ml.)	0.36	0.36	0.24	4.96	2.16	0.18
Total cholesterol (mg./100 ml.)	190	189	154	129	180	189
Alkaline phosphatase (units)	2.1	1.5	1.3	8.1	3.6	5.4
Thymol turbidity (units)	1.3	2.2	1.3	15.3	3.9	1.9
Thymol flocculation	(−)	(−)	(−)	+++	(−)	(−)
Cephalin cholesterol	(−)	(−)	(−)	+++	(−)	(−)
Colloidal red	(−)	(−)	(−)	+++	+	(−)
Zinc turbidity (units)	7.1	7.1	8.3	10.8	14.3	17
Prothrombin complex (per cent normal)	100	100	100	100	100	100
Labile factor (per cent normal)	100	100	100	100	100	100
Serum iron (μg./100 ml.)	114	106	85	178	150	120
Oxaloacetic transaminase (units/ml.)	42	33	32	293	51	40
Pyruvic transaminase (units/ml.)	36	26	23	545	63	34
Bromsulphalein (per cent retention 30 min.)	4	4		43		
Serum albumin (gm./100 ml.)	3.5	3.45		3.22	3.15	2.71
Serum gamma globulin (gm./100 ml.)	1.5	1.62		2.02	1.72	1.95

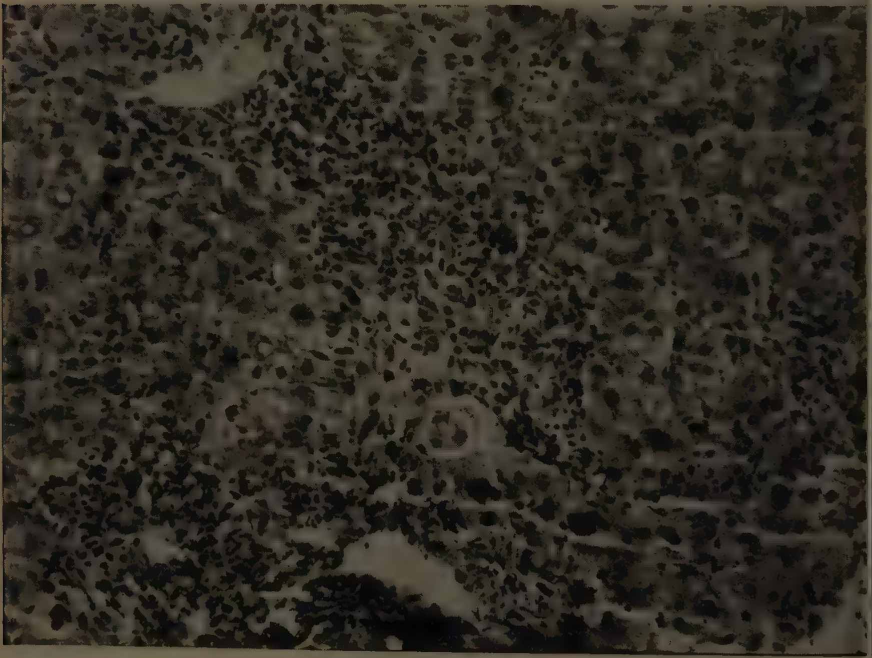

FIGURE 3. Serial histological studies of patient No. 42, who developed hepatitis. After 96 days of treatment: clear-cut hepatocellular damage, marked inflammatory infiltration in the portal spaces (*below*), diffuse and in relation to the central vein (*upper left*). Hematoxylin and eosin stain. ×240.

period preceding the discovery of hepatitis 4 laboratory controls and 1 liver biopsy were done; the results of the laboratory tests are shown in TABLE 4, and the 2 biopsies are shown in FIGURES 1 and 2.

On August 5 the patient appeared slightly jaundiced, and the liver was barely palpable below the costal margin; he had noticed malaise and mild anorexia during the preceding 5 days. Treatment with Marsilid was immediately discontinued. The results of the biochemical examination are shown in TABLE 4. Three days later a liver biopsy was performed (FIGURE 3). Hepatitis in this patient followed a mild course. Two subsequent laboratory controls are shown in TABLE 4.

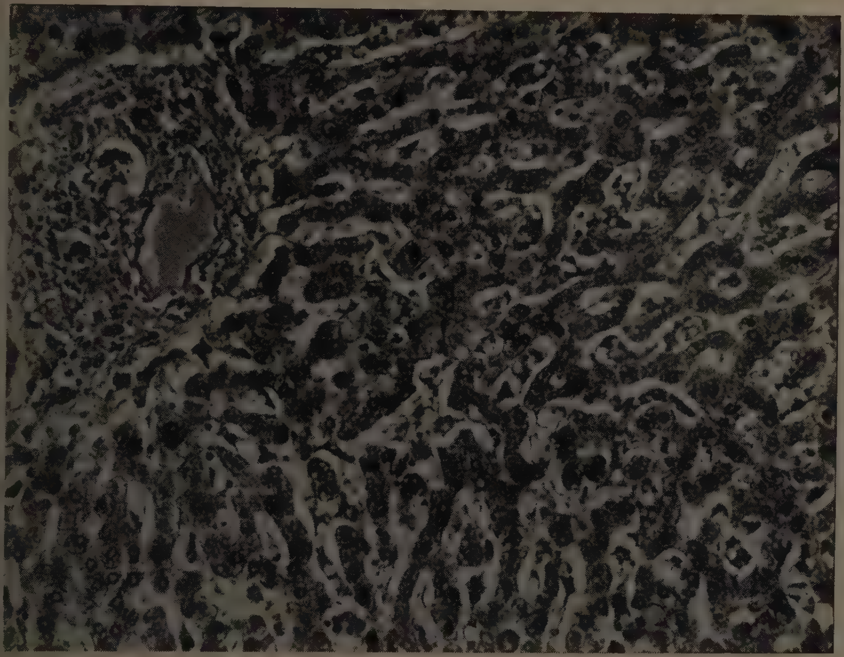

FIGURE 4. Serial histological studies in patient No. 42, who developed hepatitis. Post-mortem examination 30 days after the clearing of the jaundice: disappearance of the hepatitis picture, marked edema of the Diese spaces that could be attributed to agonical (suffocational) or post-mortem changes. Hematoxylin and eosin stain. ×240.

Five days after the last laboratory examination that yielded normal results, the patient committed suicide on October 5 during an exacerbation of his mental disturbance. At post-mortem examination the liver weighed 1800 gm., its surface was smooth, and no distortion of the normal architecture was shown at the cut. The histological aspect is shown in FIGURE 4.

The abnormal Bromsulphalein retention and the increase in the standard deviation of the mean of some of the tests at the 90-day control are explained by the case of hepatitis. Two other aberrations, namely, the increased percentage of positive results of the thymol flocculation and the colloidal red test,

TABLE 5

HISTOLOGICAL CHANGES OF THE LIVER IN PATIENTS TREATED WITH MARSILID IN WHOM HEPATIC BIOPSIES WERE PERFORMED

			Hepatic cell changes									Exudates					Proliferation							
			Nuclear					Cytoplasmatic																
Case number	Days of treatment	Global appreciation	Vacuolization	Cariolysis or cariorexis	Hyperchromia	Mitosis	Irregularities	Cloudy swelling	Microvacuolar degeneration	Fatty infiltration	Parenchymal distortion	Global appreciation	Portal	Diffuse	Neutrophils	Small round cells	Global appreciation	Histiocytes	Fibroblasts	Fibrosis	Vascular sclerosis	Periductal fibrosis	Pseudoductal proliferation	Kupffer cells hyperplasia
1	29	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
4	99	—	—	—	—	—	—	—	—	—	—	+	+	—	—	+	+	+	+	—	—	—	—	+
6	89	+	+	—	—	—	—	+	—	—	—	+	+	—	—	+	+	+	+	—	—	—	+	—
10	74	+	+	—	—	++++	+	—	—	+	—	+	+	+	+	+	+	+	—	—	—	—	—	++
12	80	—	—	—	—	—	—	—	—	—	—	+	+	+	—	+	+	+	+	—	—	+	—	+
12	150	+	—	—	+	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+
17	89	+	+	+	—	—	—	—	—	++	—	++	++	—	—	++	++	++	—	—	—	—	—	—
18	55	+	—	—	—	—	—	+	+	—	—	++	++	—	+	++	++	++	+	+	—	+	—	—
18	150	+	—	—	—	—	—	+	—	—	+	+++	+++	—	—	+++	++	++	++	+	+	—	—	—
22	4	++	—	—	++	+++	++	+	++	—	+	++++	++++	+	++	+++	+++	+++	+++	—	—	—	+++	+
22	92	—	—	—	—	—	—	—	—	—	—	+	+	—	—	+	+	—	+	+	—	—	—	—
26	4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
26	90	—	—	—	—	—	—	—	—	—	—	+	+	—	—	+	+	+	+	+	—	—	—	—
31	0	—	—	—	—	—	—	—	—	—	—	+	+	—	—	+	+	+	—	—	—	—	—	—
31	97	+	—	—	—	—	—	+	—	—	—	+	+	—	—	+	+	+	+	+	—	—	—	—
35	0	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	+	+	—	—
35	70	+	+	—	—	—	—	—	—	—	—	+	+	—	—	+	+	+	+	+	—	—	—	—
36	34	+	+	—	+	+	—	—	—	—	—	++	++	—	—	++	+	+	+	—	—	—	—	—
39	7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
39	48	+	—	—	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
40	0	—	—	—	—	—	—	—	—	—	—	+	+	—	—	+	+	+	+	—	—	—	+	—
40	70	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
42	0	+	+	+	—	+++	+	+	—	—	—	+	+	+	+	++	+	++	—	—	+	—	—	—
42	73	+	+	—	—	—	—	+	—	—	—	+	+	—	—	+	++	—	+	++	+++	+	—	—
42	96	+++	+	+	+	++	+++	+++	—	—	+	+++	+++	++	+	+++	++	+++	++	+	+++	—	+	++

Symbols: +, slight; ++, moderate; +++, moderately marked; ++++, marked.

cannot be linked to the occurrence of hepatitis. In the control performed at 110 days, the last-mentioned abnormalities of the thymol flocculation and colloidal red are also present. The rest of the laboratory tests performed were normal. Only 1 case (No. 29) showed elevation of the prompt direct bilirubin to 0.31 mg., with a normal total value of 0.72.

The study of the histopathological aspects of the liver biopsies is summarized in TABLE 5. It may easily be observed that most of the biopsies were normal or presented only slight alterations. Moreover, the pathological changes observed do not correlate with the treatment with Marsilid. In some patients clear-cut changes present at the beginning of treatment disappeared during its continuation (FIGURES 5 and 6). In others, mild abnormalities appeared. The glycogen content and the protein granules of the liver cells were normal in all patients; in none of them was hepatic cell necrosis or eosinophilic exudate present. Most of the cases showed deposits of pigment of different types in the liver cells, but this condition was also present in the pretreatment biopsies.

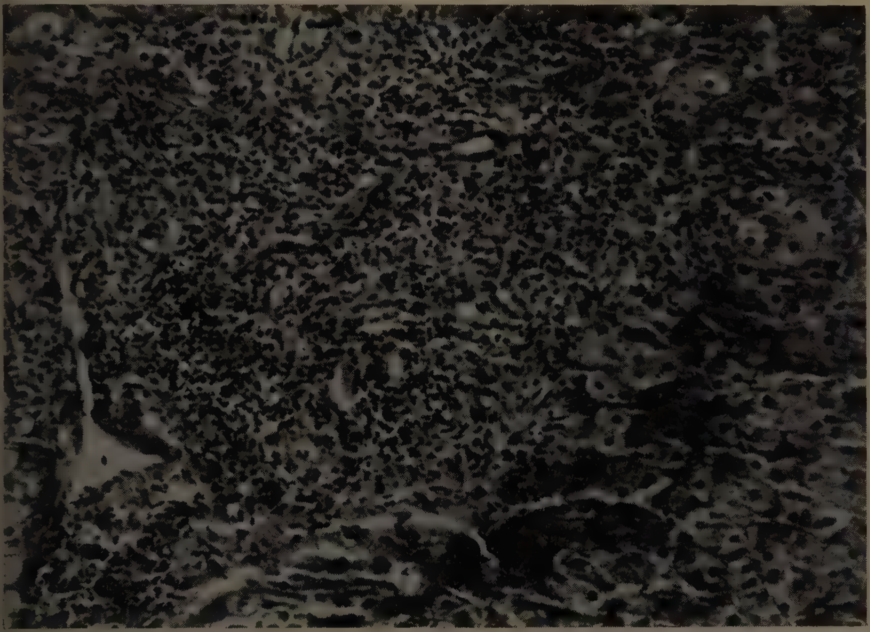

FIGURE 5. Hepatic biopsy in patient No. 22. After 4 days of Marsilid therapy: very marked parenchymal changes (*upper left*) and portal infiltration (*lower right*). Hematoxylin and eosin stain. ×240.

The results of the serial laboratory tests in the cardiac patients are summarized in TABLES 6 and 7. No liver biopsies were performed in this group. Analysis of the results shows that no abnormalities were introduced by the Marsilid treatment. Moreover, in some of the tests a reduction of the percentage of abnormal results was observed.

Discussion

The 1 case of hepatitis observed in the total number of 69 patients was indistinguishable from acute viral hepatitis. The laboratory findings and histological picture were those of hepatocellular jaundice, and the patient completely recovered from the disease. It is impossible to affirm that the jaundice was toxic in origin, since hepatitis is prevalent in Chile.

It is interesting to note that a laboratory control performed in this patient 14 days before the first symptoms appeared was normal, as was the examination carried out before starting treatment with iproniazid. These findings render valueless the liver tests in common use in the screening of patients to be treated or in the early detection of those who will develop hepatitis. It is also of interest that the histological abnormalities found at the onset of treatment diminished steadily during Marsilid therapy. Only when hepatitis developed did its classic picture appear in the liver section.

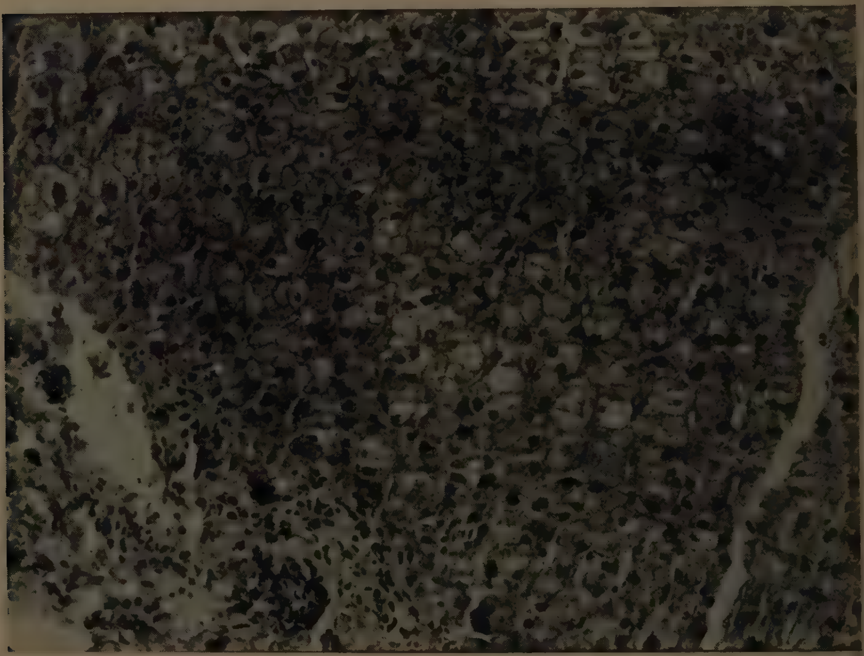

FIGURE 6. Hepatic biopsies in patient No. 22. After 92 days of Marsilid therapy: disappearance of the parenchymal changes and marked regression of the portal infiltration (*lower left*). Hematoxylin and eosin stain. ×240.

In the psychiatric group the few abnormalities found during the treatment with iproniazid relating to prompt direct and total bilirubin are difficult to interpret and cannot with certainty be attributed to the use of the drug. Marsilid therapy was continued with no deleterious effects in some patients with this abnormality.

The flocculation tests showed changes that could not be related to the length

TABLE 6

SERIAL HEPATIC TESTS ON 21 CARDIAC PATIENTS TREATED WITH MARSILID

Duration of therapy (days)		Test							
		Prompt direct bilirubin (mg./100 ml.)	Total bilirubin (mg./100 ml.)	Alkaline phosphatase (units)	Oxaloacetic transaminase (units/ml.)	Pyruvic transaminase (units/ml.)	Bromsulphalein (per cent retention)	Prothrombin complex (per cent of normal)	Labile factor (per cent of normal)
Control	No. of obser.	21	21	20	21	21	18	16	16
	Av. ± S.D.	0.10 ± 0.05	0.31 ± 0.1	4.4 ± 0.9	39.5 ± 6	29 ± 4.8	8.3 ± 3	91 ± 10	91 ± 10
	No. abnormals	0	0	1	0	0	5	2	2
	Per cent abnormals	0	0	5	0	0	27.7	12.5	12.5
10	No. of obser.	19	19	19	19	19		15	15
	Av. ± S.D.	0.09 ± 0.05	0.30 ± 0.14	4.6 ± 1.4	39 ± 5	29 ± 5.5		92 ± 8	92 ± 8
	No. abnormals	0	0	4	0	0		0	0
	Per cent abnormals	0	0	21	0	0		0	0
20	No. of obser.	10	10	10	9	9		8	8
	Av. ± S.D.	0.13 ± 0.05	0.31 ± 0.1	4.3 ± 1.4	39.7 ± 9	30 ± 9.7		88 ± 11	88 ± 11
	No. abnormals	0	0	1	1	0		1	1
	Per cent abnormals	0	0	10	11.1	0		11.3	11.3
30	No. of obser.	18	18	18	18	18	14	16	16
	Av. ± S.D.	0.14 ± 0.05	0.37 ± 0.1	4 ± 1.4	42 ± 10	31 ± 7	7.7 ± 1	93 ± 9	93 ± 9
	No. abnormals	0	0	2	2	0	2	1	1
	Per cent abnormals	0	0	11	11	0	14.3	6.2	6.2
50	No. of obser.	14	14	14	14	14		10	10
	Av. ± S.D.	0.1 ± 0.04	0.31 ± 0.16	4.6 ± 1.5	41 ± 8.8	27 ± 5.9		98 ± 4	98 ± 4
	No. abnormals	0	0	3	1	0		0	0
	Per cent abnormals	0	0	21.3	7.1	0		0	0
70	No. of obser.	8	8	8	8	8		8	8
	Av. ± S.D.	0.11 ± 0.03	0.28 ± 0.09	4 ± 1.7	35 ± 4.7	30 ± 3		100 ± 0	100 ± 0
	No. abnormals	0	0	2	0	0		0	0
	Per cent abnormals	0	0	25	0	0		0	0
90	No. of obser.	8	8	8	8	8	8	8	8
	Av. ± S.D.	0.09 ± 0.04	0.29 ± 0.13	4.9 ± 1.8	35 ± 4	30 ± 3.6	6.5 ± 2	97 ± 1.5	97 ± 1.5
	No. abnormals	0	0	1	0	0	1	0	0
	Per cent abnormals	0	0	12.5	0	0	12.5	0	0

TABLE 7

SERIAL HEPATIC TESTS ON 21 CARDIAC PATIENTS TREATED WITH MARSILID

Duration of therapy (days)		Test							
		Thymol turbidity (units)	Thymol flocculation	Cephalin flocculation	Colloidal red	Zinc turbidity (units)	Serum albumin (gr./100 ml.)	Gamma globulin (gr./100 ml.)	Serum iron (μg./100 ml.)
Control	No. of obser.	21	21	21	21	21	21	21	21
	Av. ± S.D.	4.3 ± 2	—	—	—	12.3 ± 4	3.6 ± 0.5	1.4 ± 0.3	98 ± 37
	No. abnormals	5	1	0	1	3	3	0	1
	Per cent abnormals	24	4.8	0	4.8	15	15	0	4.8
10	No. of obser.	19	19	19	19	19			19
	Av. ± S.D.	3.8 ± 1.9	—	—	—	11 ± 4.5			98 ± 27
	No. abnormals	3	1	1	1	3			1
	Per cent abnormals	15.8	5.3	5.3	5.3	14.3			5.3
20	No. of obser.	10	10	10	10	10			10
	Av. ± S.D.	4.2 ± 2.2	—	—	—	14.5 ± 4			108 ± 31
	No. abnormals	1	0	2	1	2			1
	Per cent abnormals	10	0	20	10	20			10
30	No. of obser.	18	18	18	18	18	18	18	18
	Av. ± S.D.	4.3 ± 2.1	—	—	—	11 ± 3.4	3.7 ± 6.4	1.4 ± 0.3	108 ± 34
	No. abnormals	2	0	3	2	1	0	0	2
	Per cent abnormals	11	0	16.6	11	5.5	0	0	12.4
50	No. of obser.	14	14	14	14	14			14
	Av. ± S.D.	4 ± 2.5	—	—	—	10.5 ± 4.4			98 ± 37
	No. abnormals	1	1	2	1	2			1
	Per cent abnormals	7.1	7.1	14.2	7.1	14.2			7.1
70	No. of obser.	8	8	8	8	8			8
	Av. ± S.D.	4 ± 1.4	—	—	—	9.7 ± 2.1			97 ± 17
	No. abnormals	0	0	0	0	0			0
	Per cent abnormals	0	0	0	0	0			0
90	No. of obser.	8	8	8	8	8	8	8	8
	Av. ± S.D.	4.6 ± 1.4	—	—	—	11 ± 1.6	3.4 ± 0.2	1.6 ± 0.2	112 ± 16

of Marsilid treatment, since they were within a range similar to those we had observed in apparently normal subjects controlled for long periods. Moreover, the number of abnormal results observed at the different controls was not always obtained in the same group of patients; furthermore, at a given period one patient could be positive to one test and negative to others.

More specific tests, such as Bromsulphalein and transaminase, were definitely abnormal only in the case of hepatitis. In the other 4 patients in whom 1 of the transaminase tests was abnormal the values were only 2 or 3 units over our upper normal limit.

The results obtained with the study of prothrombin complex and labile factor show that these two tests are not useful in this type of study, as both were normal in our case of hepatitis.

Other tests, such as alkaline phosphatase, protein fractions, and serum iron, may show the development of hepatitis, as in our case. Nevertheless, when considered for the whole group, we may see that they do not contribute to a better understanding of the problem when the proportion of liver damage is low.

The case of hepatitis and the alterations of the serum bilirubin appeared in the psychiatric group, but no abnormalities were found in the cardiac patients. However, the size of the groups studied does not permit us to recognize different behavior between the psychiatric and cardiac patients under the Marsilid treatment.

Slight abnormalities were found in the majority of the liver biopsies performed but, in the cases with repeated biopsies, these were present before initiation of treatment. In some patients the histological picture improved during treatment, which is even more difficult to interpret; in this respect, it is interesting to emphasize that the patient who showed more abnormalities in the first biopsy had a normal second one during therapy. It thus appears that clear-cut pretreatment histological abnormalities of the liver do not indicate that hepatic damage will develop during therapy with Marsilid.

Summary and Conclusions

A serial clinical, biochemical, and biopsy study of the liver in 69 patients treated with iproniazid is presented.

In the entire group we observed only one case of hepatitis that followed a benign course. This case showed the laboratory and biopsy findings of hepatocellular jaundice, and it is impossible to ascertain its toxic origin.

The series of tests performed do not appear to be useful in the screening or in the follow-up of patients treated with a potentially toxic drug that can produce hepatocellular damage. From the battery of tests employed, those apparently most useful are bilirubin, transaminases, and Bromsulphalein.

The histological liver abnormalities do not seem to be a contraindication to the use of Marsilid and, in some instances, they disappear during its use.

It is our impression that Marsilid cannot be considered as a true hepatotoxic drug.

References

1. Davis, W. A. 1958. The history of Marsilid. J. Clin. Exptl. Psychopathol. Quart. Rev. Psychiat. Neurol. **19:** Suppl. 1, 1.

2. Bosworth, D. M., H. A. Wright & J. W. Fielding. 1952. The treatment of bone and joint tuberculosis: effect of 1-isonicotinyl-2-isopropylhydrazine. A preliminary report. J. Bone and Joint Surg. **34A:** 761.
3. Bosworth, D. M., H. A. Wright, J. W. Fielding & H. J. Wilson, Jr. 1953. The use of iproniazid in the treatment of bone and joint tuberculosis, J. Bone and Joint Surg. **35A:** 577.
4. Bosworth, D. M., J. W. Fielding, L. Demarest & M. Bonaquist. 1955. Toxicity to iproniazid (Marsilid) as it affects osseous tuberculosis. Quart. Bull. Sea View Hosp **16:** 134.
5. Scherbel, A. L. 1957. The effect of isoniazid and of iproniazid in patients with rheumatoid arthritis. Cleveland Clin. Quart. **24:** 90.
6. De Verteuil, R. L. & H. E. Lehmann. 1958. Therapeutic trial of iproniazid (Marsilid) in depressed and apathetic patients. Can. Med. Assoc. J. **78:** 131.
7. Settel, E. 1958. Marsilid for elderly persons. J. Clin. Exptl. Psychopathol. & Quart. Rev. Psychiat. Neurol. **19:** Suppl. 1, 98.
8. Zetzel, L. & H. Kaplan. 1958. Liver damage concurrent with iproniazid administration. New Engl. J. Med. **258:** 1209.
9. Frantz, A. G. 1958. Fatal jaundice associated with iproniazid (Marsilid) therapy Report of a case. J. Am. Med. Assoc. **167:** 987.
10. Ducci, H. 1949. Ictericias y laboratorio. Publicación de la Universidad de Chile Santiago, Chile.
11. Kunkel, H. C. 1947. Estimation of alterations of serum gamma globulin by a turbidimetric technique. Proc. Soc. Exptl. Biol. Med. **66:** 217.
12. Ducci, H. & R. Katz. 1952. Estado actual del problema de la coagulación y método clínicos para su estudio. Rev. Méd. Chile. **80:** 144.
13. Ducci, H., A. Spoerer & R. Katz. 1952. Serum iron in liver disease. Gastroenterology. **22:** 52.
14. Katz, R., H. Ducci, M. Velasco & E. Cerutti. 1958. Las transaminasas en las enfermedades hépatobiliares. Rev. Méd. Chile. **86:** 25.

Discussion of the Paper

Jesse Bollman (*Mayo Clinic, Rochester, Minn.*): We should thank Kat and his associates for their very thorough work on possible liver damage i patients receiving therapeutic amounts of iproniazid. This study clearly establishes the fact that man does not react differently to iproniazid than d laboratory animals. It is especially interesting that the one patient who di develop hepatitis during iproniazid therapy was not detected before the usua signs of hepatitis appeared, despite intensive study of his liver function. Thi fact alone should prevent one from placing very great reliance on liver-function tests as a screening procedure for detecting incipient liver damage during iproniazid therapy.

It would also be well to call attention to the fact mentioned by Katz tha minor variations in hepatic function tests do occur fairly frequently in hospitalized patients. Interpretation of the single-test variations is extremel difficult, and certainly at the present they cannot be correlated with any othe evidence of hepatic insufficiency.

I congratulate Katz and his collaborators for this thorough and well-presented investigation.

SOME PRELIMINARY CLINICAL AND BIOCHEMICAL ASPECTS OF LIVER FUNCTION IN PATIENTS RECEIVING IPRONIAZID

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Jaundice resulting from drug toxicity may be of the hepatocellular, "cholangiolitic," or even hemolytic type. Knowledge of the type of jaundice that occurs as a drug reaction may be useful in distinguishing it from other hepatobiliary disease. Thus, the characteristic cholangiolitic features of chlorpromazine jaundice usually permit a distinction from coincident viral hepatitis. Furthermore, the selection of the laboratory procedures that may make possible the early detection of hepatic toxicity depends on the type of abnormal hepatic function observed in the jaundiced patient.

Accordingly, the occurrence of jaundice in a small fraction of patients receiving iproniazid (Marsilid*) has made necessary the study of biochemical features of the syndrome and evaluation of the incidence of hepatic dysfunction in patients receiving the drug.

Methods And Material

Eighty-six patients who developed jaundice while receiving iproniazid were studied, data on 82 of these having been obtained from protocols submitted to the manufacturer by individual physicians. The other 4 patients were from the Chicago, Ill. area. Many of the patients had received other drugs or had had venipunctures for various reasons. All had jaundice while receiving iproniazid. Serum bilirubin determinations were available in 78 patients, thymol turbidity values in 56, cephalin flocculation results in 71, and alkaline phosphatase levels in 72. These procedures were performed in the individual laboratories serving the responding physicians. The results were considered abnormal when the thymol turbidity was above 4 units, the cephalin flocculation 3 or 4 plus at 48 hours, and the alkaline phosphatase greater than 4 Bodansky units. For the sake of uniformity in analyzing the data, alkaline phosphatase values reported in King Armstrong units have been converted to Bodansky units (B.U.) by dividing by a factor of 1.8.[1] Glutamic-oxaloacetic transaminase (GOT) determinations were available in 50 patients, and glutamic-pyruvic transaminase (GPT) in 22. All but 18 transaminase determinations were performed in the individual laboratories. Sera from these 18 were submitted to our laboratory for enzyme determinations and were studied for GOT and GPT activity by the spectrophotometric method of Karmen;[2] 11 sera were studied for lactic dehydrogenase (LD) activity by the method of Zimmerman and Weinstein,[3] 9 sera for aldolase activity by a modification of the method of Sibley and Lehninger,[4] and 9 for isomerase activit by a modification of the method of Bodansky.[5]

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* Hoffmann-La Roche.

Results

Jaundice was intense, 45 per cent of the patients having bilirubin level greater than 20 mg. per cent, and values as high as 60 mg. per cent were observed. In three fourths of the patients the direct-reading fraction comprise 40 to 65 per cent of the total bilirubin. In all but 1 of the remainder, 65 t 75 per cent of the total bilirubin was direct, and in only 1 patient was ther less than 40 per cent of the total direct-reading bilirubin (FIGURE 1).

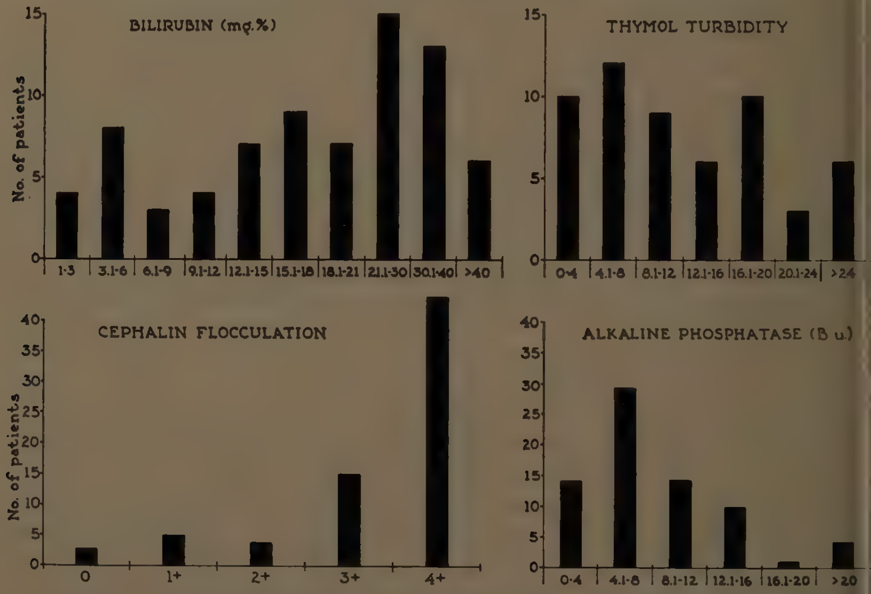

FIGURE 1. Range of abnormality of serum bilirubin, thymol turbidity, and alkaline pho phatase in 86 patients with "iproniazid jaundice."

Abnormal thymol turbidity values were observed in 83 per cent of the grou (FIGURE 1). The degree of abnormality ranged from 4 to 41 units. Th cephalin flocculation test yielded abnormal results in 85 per cent of the patients.

Alkaline phosphatase values were abnormal in 81 per cent (58 of 72) of th patients studied. In 40 per cent of the total group, values between 4 and B.U. were observed, while in one third of the patients these values range from 8 to 16 units. There were 5 patients (7 per cent) with values above 1 units, and 4 of these were above 20 units (FIGURE 1).

TABLE 1 lists other biochemical abnormalities observed. Serum iron leve were elevated in the 6 patients studied. Prolongation of prothrombin tim was observed in 21 of 24 patients, and cholesterol esters were below 60 pe cent of the total in 9 of the 13 patients studied. Serum albumin levels we below 3 gm. per cent in 8 of 27 patients. Serum globulin levels were elevate (above 3 gm.) in 46 per cent. Only 1 patient had a globulin level above 4 gm. per cent.

It is of interest to compare the laboratory features of "iproniazid jaundice" and viral hepatitis (TABLE 2). The incidence of abnormality judged by various procedures shown in TABLE 2 is similar for the 2 groups. As may be seen in FIGURE 2, however, the degree of hyperbilirubinemia was greater in the patients with iproniazid jaundice than in those with hepatitis, which was true for all age groups.

TABLE 1
INCIDENCE OF ABNORMALITY OF SERUM IRON, PROTHROMBIN TIME, CHOLESTEROL, AND PROTEINS

	Number of abnormal patients	Percentages of patients tested
Increased serum iron	6	100
Prothrombin time	21	87
Cholesterol esters 60 per cent	9	70
Serum albumin <3 gm. per cent	8	29
Serum globulin >3 gm. per cent	11	46
Serum globulin >4 gm. per cent	1	4

TABLE 2
COMPARISON OF INCIDENCE OF SERUM BIOCHEMICAL ABNORMALITIES IN 86 PATIENTS WITH IPRONIAZID JAUNDICE AND 112 PATIENTS WITH VIRAL HEPATITIS

	Iproniazid (percentages)	Viral hepatitis (percentages)
Bilirubin	100	97
Thymol turbidity	83	92
Cephalin flocculation	82	91
Alkaline phosphatase	81	92
GOT	93	100
GPT	100	100
Aldolase	↑	↑
Isomerase	↑	↑
Serum iron	100	70
Globulin	46	78

An attempt has been made to classify the patients into the categories of hepatocellular or cholangiolitic jaundice (FIGURE 3). Patients were considered to have cholangiolitic jaundice if the alkaline phosphatase level was above 15 B.U.* and the thymol turbidity and cephalin flocculation tests yielded normal results. There were only 2 patients in this category. In the hepatocellular group, which was characterized as having alkaline phosphatase values below 15 B.U. and 1 or both flocculation tests yielding abnormal results, there were 59 patients. Eleven patients were considered to have a mixed pattern, being neither clearly cholangiolitic nor hepatocellular. Five of these had alkaline phosphatase values in the cholangiolitic range, but also abnormal flocculation

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* In our laboratory 95 per cent of patients with viral hepatitis have serum alkaline phosphatase levels below 15 B.U.

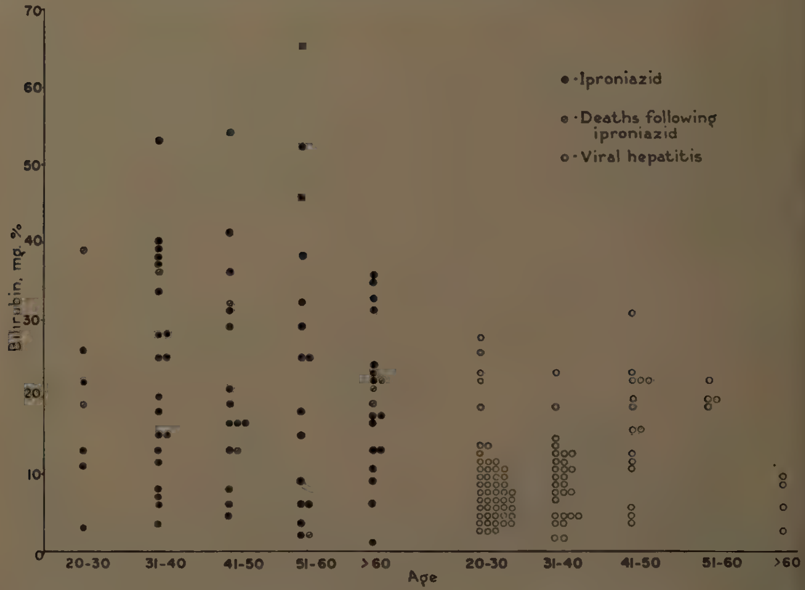

FIGURE 2. Serum bilirubin levels in 86 patients with "iproniazid jaundice" and 112 pa tients with viral hepatitis, related to age groups.

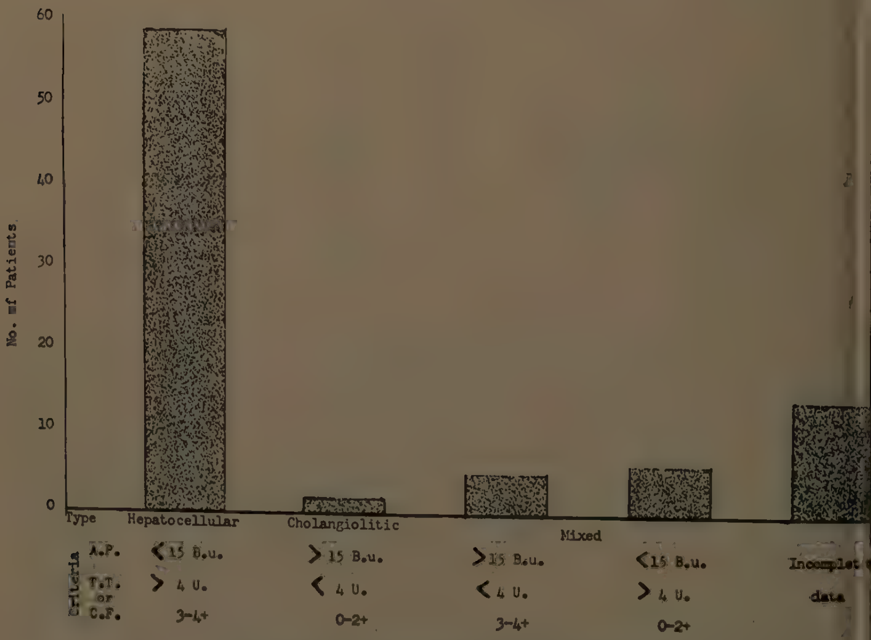

FIGURE 3. Types of jaundice observed in 86 patients with "iproniazid jaundice."

tests. The other 6 patients had normal or only mildly elevated alkaline phosphatase levels, but with normal flocculation tests. In 14 patients the data were incomplete.

The results of the serum enzyme determinations are shown in FIGURE 4. Only 4 of the 50 patients studied had normal GOT levels, and 60 per cent of the group had levels above 300 units. In general, the levels below 300 units were from patients during the first 3 days of jaundice (3 patients), late in convalescence (4 patients) or in mild jaundice (4 patients) or cholangiolitic jaundice (2 patients). However, there were 7 patients with deep hepatocellular jaundice with only moderate GOT elevations (100 to 300 units). Values above 2000 units were observed in some patients.

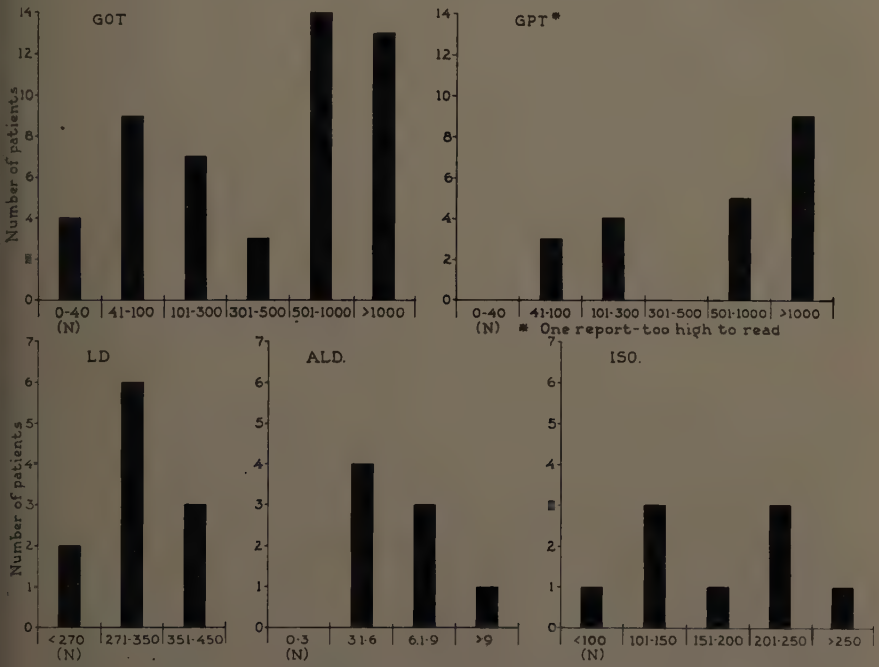

FIGURE 4. Range of abnormality of serum enzymes in patients with "iproniazid jaundice." Abscissa figures are in Karmen units (GOT and GPT), micromoles of DPN reduced per 100 ml. serum (L.D.) millimicromoles of substrate converted/ml. serum/minute (aldolase and isomerase).

In the 17 patients with simultaneous GOT and GPT determinations, the 2 transaminase levels were compared (TABLE 3). In 10 patients the GPT value was elevated to a greater degree than the GOT, in 3 patients the reverse was true and, in 4 patients, the 2 transaminase levels were approximately equal (less than 25 units difference).

The incidence and degree of elevation of the serum LD, aldolase, and isomerase also are shown in FIGURE 4. The LD levels were moderately elevated in 8 of the 11 patients studied. All 9 patients with aldolase determinations showed elevated levels, and 8 of the 9 isomerase levels also were elevated. No attempt has been made to correlate these few values with the abnormalities of GOT and GPT.

Liver biopsy specimens were obtained in 3 of the 4 patients individually studied. All 3 showed necrosis, inflammation, and cholestasis; one of these cases is illustrated in FIGURE 5. The serial biochemical data from this patient are shown in FIGURES 6 and 7. This 69-year-old white female had had a myocardial infarction and a cerebral thrombosis 5 years and 1 year prior to admission, respectively. From the time of the cerebrovascular accident until the onset of jaundice she had had numerous venipunctures for prothrombin activity determination for the "control" of anticoagulant therapy. During the 6 weeks before jaundice appeared, iproniazid was administered for treatment of depression. The dose was 75 mg./day for 5 days, 50 mg./day for 15 days and, thereafter, 25 mg./day. Four weeks after initiation of iproniazid administration, anorexia appeared and, 2 weeks later, jaundice was observed. On admission to the hospital the patient was deeply jaundiced and the liver was palpated 5 cm. below the right costal margin. During the 2 weeks after admission there was progressive deepening of jaundice; a "flapping tremor" followed by hepatic coma developed, and the patient died on the fourteenth hospital day.

TABLE 3

COMPARISON OF SIMULTANEOUSLY DETERMINED GOT AND GPT

	No. patients
GPT > GOT	10
GPT < GOT	3
GPT = GOT*	4

* When the 2 determinations were separated by less than 25 Karmen units they were considered equal.

The initial laboratory data (FIGURES 6, 7) are of interest in that strikingly elevated GOT and GPT levels were present, although the thymol turbidity and cephalin flocculation results were normal. Five days later the studies showed a pattern classic for hepatocellular jaundice.

Serial Studies in Patients Receiving Iproniazid

A total of 70 patients was studied (TABLE 4). In 62 of them the tests of liver function included thymol turbidity, cephalin flocculation, bilirubin, alkaline phosphatase, and protein levels of the serum. In 17 of these GOT and GPT tests were performed. In 8 patients only the transaminases were determined. No significant impairment of liver function could be demonstrated in this group of patients. In 55 patients the results of liver function tests remained normal throughout periods as long as 12 months of observation. The daily dose of the drug varied from 25 to 150 mg. In 11 patients abnormality of 1 or more tests of hepatic function were present before therapy or appeared irregularly during therapy without apparent relationship to the drug

FIGURE 5. Photomicrograph of liver biopsy in fatal case of "iproniazid jaundice" showing necrosis, necrobiosis, and bile casts. Laboratory data of this patient are shown in FIGURES 6 and 7.

administration or its dose. Characteristic of such minor fluctuations was the course illustrated in FIGURE 8. In 4 patients minimal abnormalities appeared during therapy and disappeared coincident with reduction of dose or discon-

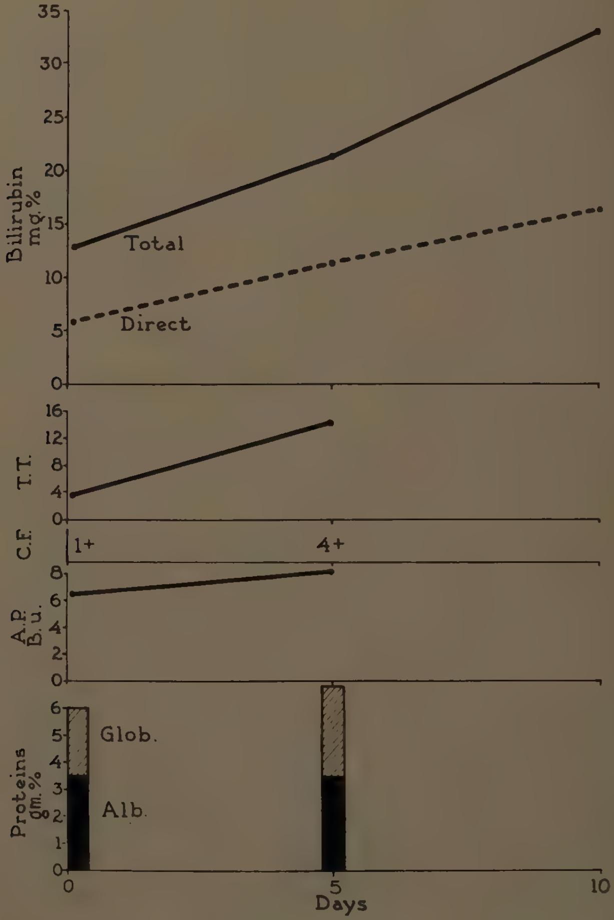

FIGURE 6. Serial levels of serum bilirubin, thymol turbidity, cephalin flocculation, alka line phosphatase and serum proteins in a patient with "iproniazid jaundice." (A biopsy o this case is shown in FIGURE 5.)

tinuance of the drug. The course of the patient who showed this relationship most clearly is illustrated in FIGURE 9.

It may be of interest to report preliminary studies on the effects of another amine oxidase inhibitor, phenylethylhydrazine hydrochloride (W-1544). This drug was administered to 45 patients daily in doses of 25 to 100 mg. for period of 1 to 2 months. No significant change in thymol turbidity, cephalin flocculation, serum bilirubin, alkaline phosphatase, or proteins developed.

Discussion

The results of the present study suggest that the jaundice associated with iproniazid administration is usually hepatocellular in type. This is shown by the occurrence of abnormal flocculation tests, markedly elevated transaminase levels, and the histological demonstration of necrosis. Eighty-two per cent of the cases that could be analyzed had hepatocellular jaundice, 3 per cent were cholangiolitic, and the remainder were considered as mixed. Whether the patients with cholangiolitic jaundice represent a different etiology

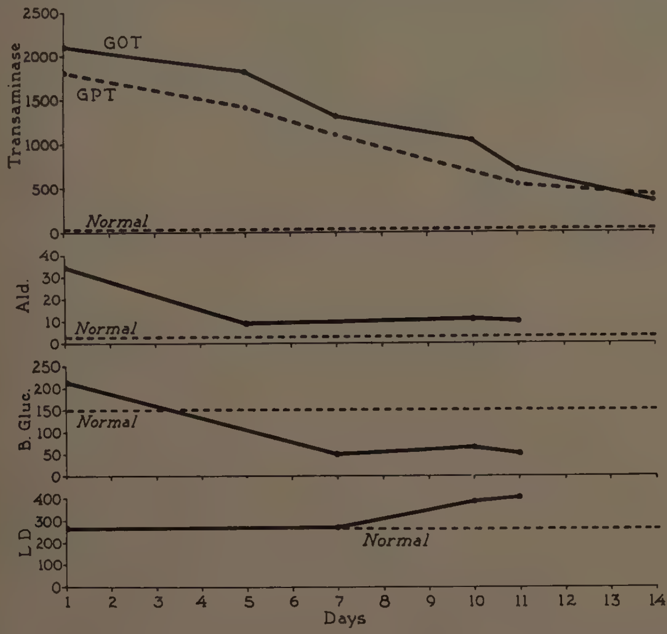

FIGURE 7. Serial levels of serum enzymes in patient with "iproniazid jaundice." See FIGURE 4 for GOT, GPT, L.D., and aldolase units of measurement. β-Glucuronidase expressed as micrograms of phenolphthalein liberated from phenolphthalein glucuronide per ml. serum.

TABLE 4

ANALYSIS OF SERIAL LIVER FUNCTION STUDIES IN PATIENTS RECEIVING IPRONIAZID

1 month	15
1 to 2 months	15
2 to 3 months	6
3 to 4 months	3
4 to 6 months	8
6 months	8

Group 1: All tests normal throughout period of drug administration (55 patients).
Group 2: Slight abnormalities occurring irregularly during period of drug administration (11 patients).
Group 3: Mild abnormality which disappeared with reduction of dose or discontinuance of drug (4 patients).

or merely a different host response cannot be determined from the available data. It seems clear that in individual patients iproniazid jaundice cannot be distinguished from the jaundice of viral hepatitis. This opinion also has been advanced by Zetzel and Kaplan[6] and by Katz and his associates elsewhere in this monograph.

Iproniazid jaundice appears to be more severe than viral hepatitis. The mortality rate in patients analyzed by Dixon (personal communication) was approximately 20 per cent. The data presented in this study show that deep

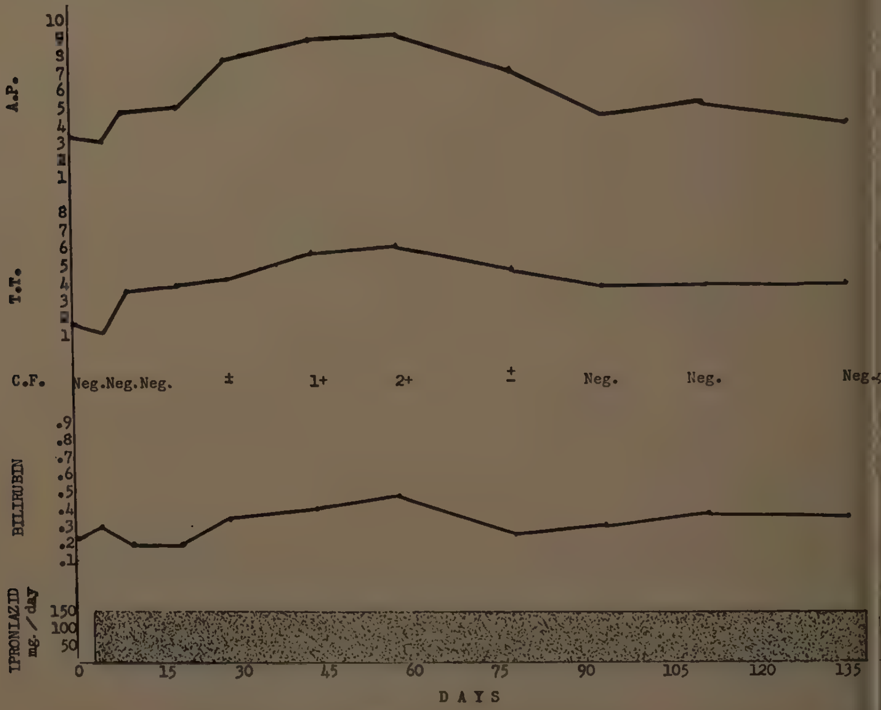

FIGURE 8. Illustration of development of slight abnormality of liver function during course of administration of iproniazid; note return to normal without withdrawal of drug.

jaundice is more frequent in patients with the drug toxicity than in those with viral hepatitis.

The incidence of jaundice in patients receiving iproniazid remains to be determined. Floody *et al.*[7] have reported an incidence of 0.02 per cent (approximately 100 cases in 500,000 treated individuals). Bosworth and his associates[8] observed 4 cases of jaundice in 142 patients (2.7 per cent) with osseous tuberculosis treated with iproniazid, but considered the jaundice to be unrelated to the drug administration. Elsewhere in these pages Katz and his co-workers report jaundice in 1 of 69 patients (1.3 per cent), while Smar *et al.*[9] noted 3 patients with jaundice in 25 who received the drug. In their presentation Scherbel and Harrison did not observe any in 921 patients who were treated with 50 mg. or less of iproniazid daily for periods varying from a few months to 5 years. Further observations are required to evaluate the true incidence.

It would appear that the tests most useful in detecting iproniazid hepatotoxicity would be those that detect early jaundice, such as the urine or serum bilirubin tests and those reflecting hepatic parenchymal abnormality, such as the flocculation tests or serum GOT and/or GPT levels. It is our impression that the serum transaminase determinations may provide evidence of hepatocellular abnormality somewhat earlier than the flocculation tests (see FIGURES 6 and 7). Since none of the 70 patients studied serially while receiving iproniazid developed jaundice, no corroboration of this thesis can be derived from our observations. Smarr *et al.*,[9] however, reporting on 25 patients receiving the drug, implied that the serum GOT had been a useful guide to early hepatotoxicity.

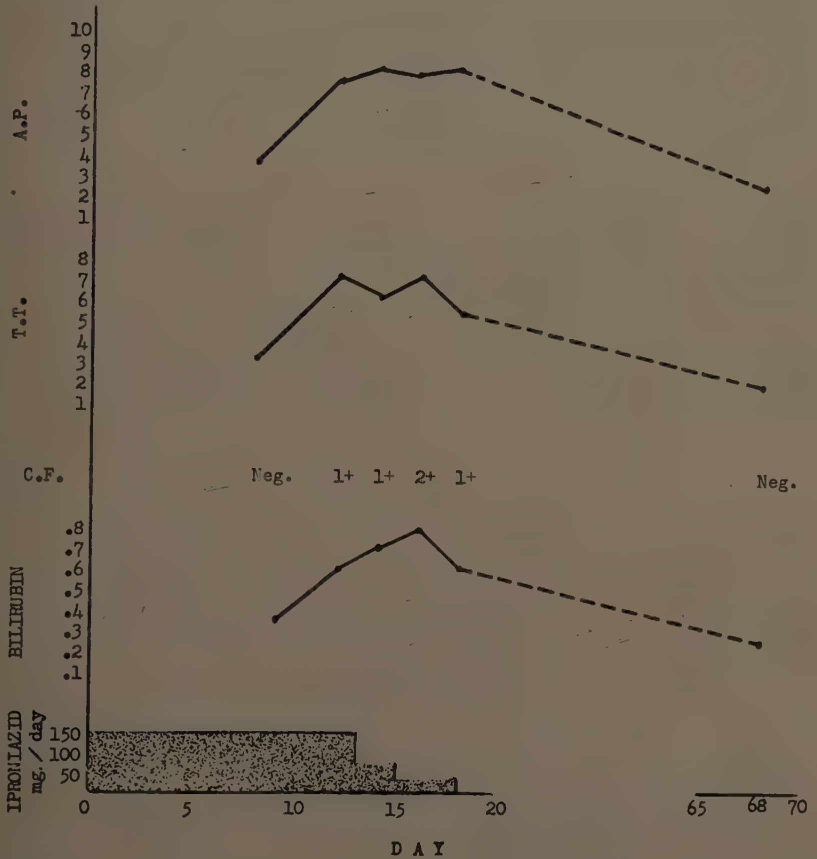

FIGURE 9. Illustration of development of slight abnormality of liver function, with return to normal on cessation of drug administration.

Thus, it would seem reasonable, at this time, to recommend that screening tests for iproniazid hepatotoxicity include at least serum GOT (or GPT) and urine bilirubin. This combination should offer a sensitive and reasonably simple means of detecting early hepatocellular damage.

The mechanism by which iproniazid may produce hepatic disease is obscure. In some instances the jaundice may be the result of coincident virus (epidemic or serum) hepatitis. Nevertheless, it is generally believed that iproniazid may be hepatotoxic to some patients. The studies that Katz and his associates report elsewhere in these pages and the apparently low incidence of jaundice suggest that this is a variety of drug idiosyncracy. Whether this is the result of an abnormal metabolic pathway for the drug, of deficient enzymatic machinery to deal with its effects, or of immunological mechanisms remains to be determined.

Summary

Eighty-six patients who developed jaundice during iproniazid therapy were studied. Hepatocellular jaundice was observed in 59 and the cholangiolitic type in 2. In 11 patients the pattern was mixed and, in 14, the data were inadequate for categorization. Biopsy in 3 patients showed hepatic necrosis. It is suggested that screening tests to detect hepatotoxicity should include at least the urine bilirubin and serum transaminase(s) determination.

Serial studies in 70 patients receiving the drug for periods of 1 to 12 months failed to reveal definite evidence of hepatotoxicity.

Acknowledgments

We are grateful to Robert E. Dixon and Robert J. Floody of Hoffmann-La Roche, Inc. for providing much of the data for this study, and to H. Yellin of Mount Sinai Hospital, P. Szanto and F. Steigman of Cook County Hospital, and S. Stein of Forest Hospital, all of Chicago, Ill., and to L. Grunewald, Sheboygan, Wis., for permission to report on their patients. We also thank Paul Feldman of Warner-Lambert Pharmaceutical Company, Morris Plains, N. J., who supplied the W-1544 used in these investigations.

References

1. Gradwahl, R. B. H. 1943. Clinical Laboratory Methods and Diagnosis. 3rd ed. Mosby. I: 308. St. Louis, Mo.
2. Karmen, A., F. Wróblewski & J. S. La Due. 1955. J. Clin. Invest. **34:** 126.
3. Zimmerman, H. J. & H. G. Weinstein. 1956. J. Lab Clin. Med. **48:** 607.
4. Sibley, J. A. & A. L. Lehninger. 1949. J. Biol. Chem. **177:** 859.
5. Bodansky, O. 1954. Cancer. **7:** 1191.
6. Zetzel, L. & E. Kaplan. 1958. New Engl. J. Med. **258:** 1209.
7. Floody, R. J., R. E. Dixon & V. D. Mattia, Jr. A preliminary report—Marsilid Toxicity. To be published.
8. Bosworth, D. M., J. W. Fielding, L. Demarest & M. Bonaquist. 1955. Quart. Bull. Sea View Hosp. **15:** 134.
9. Smarr, E. R., H. Wolf & M. D. Pressman. 1958. Am. J. Psychiat. **114:** 1115.

Discussion of the Paper

Jesse Bollman (*Mayo Clinic, Rochester Minn.*): The observations of Zimmerman and his colleagues are similar to those reported elsewhere in these pages by Katz and his co-workers. No change in hepatic function could be demonstrated as resulting from iproniazid (Marsilid) therapy alone. Where hepatic changes did occur, the picture was indistinguishable from that of hepatitis. There seems to be a tendency toward extremely high serum bilirubin levels in hepatitis during iproniazid therapy. In a given case of jaundice this should alert the physician to a possible history of iproniazid administration. I am especially intrigued by this high level of serum bilirubin because it is so much higher than that occurring in experimental animals after ligation of the common bile duct or in extensive necrosis of the liver produced by hepatotoxic agents. The only means I know of producing such high levels of bilirubin in experimental animals is by the administration of toluenediamine, which produces intrahepatic obstruction, necrosis, and hemolysis.

I have tried the combination of toluenediamine and iproniazid in several dogs. In normal dogs about 80 mg. toluenediamine/kg. body weight is required to produce this high level of serum bilirubin. Smaller amounts appear ineffective except to produce some hemolysis without jaundice. In dogs receiving 10 mg./kg. of iproniazid daily for 5 to 10 days, jaundice was produced by the administration of toluenediamine in amounts of 50 mg./kg.

The histological picture of the liver in these animals appeared very similar to that of the normal animal receiving 80 mg./kg. This certainly does not represent a great sensitization of the liver due to the administration of iproniazid. It seems more likely that the difference is due to the increased red cell turnover that occurs during the administration of iproniazid, and is so clearly shown by Zbinden and Studer elsewhere in this monograph.

HEPATIC INJURY IN PATIENTS WHO HAVE RECEIVED IPRONIAZID*

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Whenever functional or structural evidence of hepatic injury appears after the exposure to drugs or other chemicals, the question of a causative relation between them and the hepatic injury arises. Moreover, the nature of the hepatic lesion becomes a problem, one approach to which is the study of the histological alterations visible in the livers of patients who develop clinical evidence of hepatic injury after exposure to the substances in question. Such studies also assist in the clinical problem of separating hepatic drug reactions from independent or intercurrent liver diseases.

This question recently arose when instances of jaundice were reported as associated with clinical and functional indications of hepatocellular injury following the intake of iproniazid (1-isonicotinyl-2-isopropylhydrazine, or Marsilid,† an amine oxidase inhibitor used in the treatment of tuberculosis and various mental disorders.[1-6] Biopsy and autopsy specimens of liver were obtained for study from clinicians or pathologists from various parts of the United States. The histological findings were correlated with the clinical picture, the dose of the drug administered, and the duration of the jaundice. The histological manifestations encountered were those usually considered characteristic of viral hepatitis, and no relationship between the dose of iproniazid and the extent of the lesion was observed.[7] In continuation of these studies, additional material was collected through the courtesy of several pathologists and clinicians and, in the following, an attempt is made to describe the salient pathological features in the liver, to trace the evolution of the disease, to emphasize the incidence of features typically found in patients in whom viral hepatitis is accepted on epidemiological grounds or by exclusion of other etiological factors and, finally, to point out similarities to the histological changes in patients who had developed jaundice after exposure to several other drugs, particularly cinchophen.

The material of this study consists of 16 autopsy and 22 biopsy specimens of patients who developed jaundice while receiving iproniazid or up to 20 days after cessation of this treatment, which was prescribed for depression in almost every instance. The material was obtained from a total number of 34 patients, in one instance 2, and in another 3, biopsy specimens being available and, in a third case, an autopsy and a biopsy specimen. In about one half of the cases various special stains were performed; in the other half only hematoxylin-eosin-stained single slides were available for study. All patients studied had jaundice; 14 showed a severe spotty necrotic hepatitis, as seen in biopsy specimens, 3 were in other stages of the same disease process, 2 of them exhibiting almost complete histological recovery after subsidence of

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* The work reported in this paper was supported in part by the Jennie Armour Memorial Fund for the Study of Hepatic Disease of The Mount Sinai Hospital, New York, N. Y.

† Hoffmann-La Roche.

jaundice. Of the fatal instances studied, 11 presented the picture of acute hepatitis with massive, submassive, or zonal necrosis and subsequent collapse; in 7 instances subacute hepatitis and, in 1 instance chronic hepatitis of the same type, both with various degrees of beginning nodular regeneration, were noted.

Observations

In the great majority of the biopsy specimens the arrangement of the liver cell plates was obscured because of their irregular direction and because of the loss of single or small groups of cells (FIGURE 1). The latter changes predominated in the centrolobular zone. The persisting liver cells exhibited great variation in shape and staining qualities from cell to cell in the acute stages. Some were ballooned because of hydropic swelling of the cytoplasm; others were smaller than normal. The cytoplasmic basophilia varied, being increased in some of the cells, particularly in large ones with multiple nuclei and nucleoli indicating regeneration. In other cells part or all of the cytoplasm was deeply acidophilic. Where this condition involved the entire cell, the nuclei were pyknotic and eventually disappeared when the cell was expelled from the liver cell plate. Finally, highly refractile "acidophilic bodies" appeared in the tissue spaces (FIGURE 2). The bile canaliculi were frequently

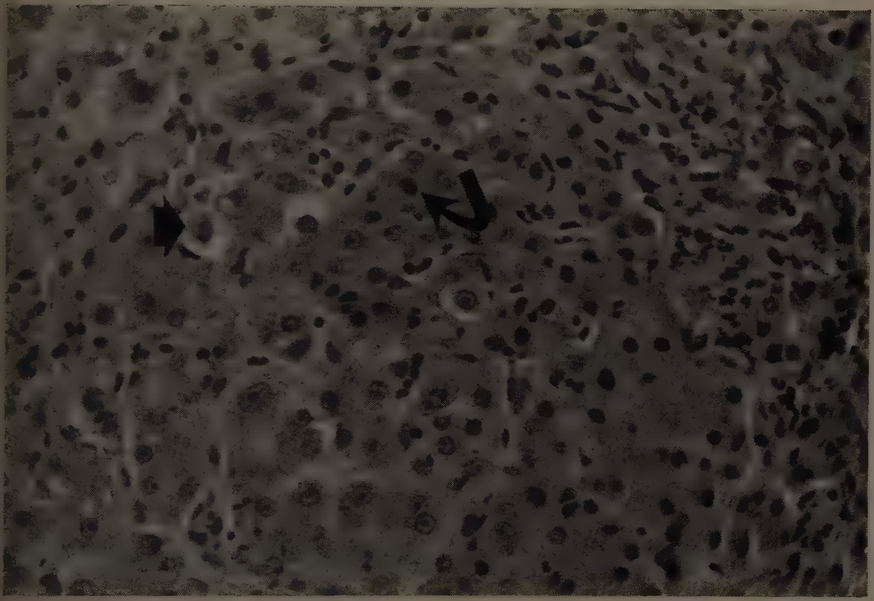

FIGURE 1. Acute spotty necrotic hepatitis in biopsy specimen of patient who had received iproniazid, showing great variations in size and staining qualities of neighboring liver cells, bile cast in dilated bile capillary (*curved arrow*), acidophilic body with rudimentary nucleus in tissue space (*straight arrow*), and interstitial inflammatory infiltration. Hematoxylin-eosin stain. ×240.

dilated and contained protein debris or bile casts. Interstitial infiltration consisted of mononuclear cells with homogeneous or granular cytoplasm that frequently contained lipofuscin pigment. Lymphocytes, plasma cells, and segmented leukocytes, many of them eosinophilic, were intermixed in varying degrees in different cases. In addition, the Kupffer cells appeared to be mobilized and protruded into the lumen. In places, especially in the centrolobular zone, the sinusoids were free of red cells because of either compression or of obstruction by debris. In the peripheral zone of the lobules, excess numbers of ductules were seen. The epithelial lining of these ductules appeared irregular because of focal aggregation of cells. The lumens varied in width, contained

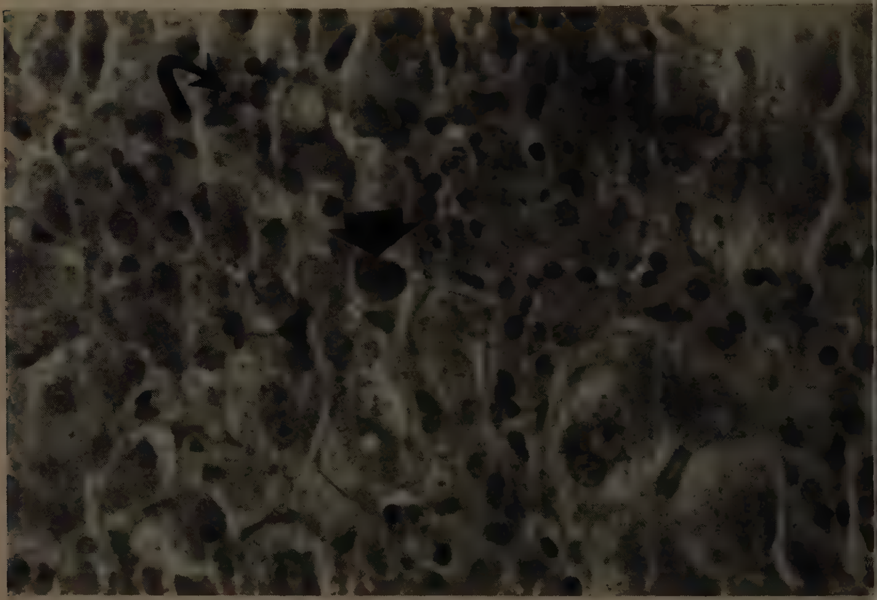

FIGURE 2. Acute spotty necrotic hepatitis in biopsy specimen of patient who had received iproniazid, showing irregular arrangement of liver cells, some of which are multinucleated considerable accumulation of interstitial cells (*curved arrow*) and anuclear acidophilic body in tissue space (*straight arrow*). Hematoxylin-eosin stain. ×480.

protein material and bile casts and, frequently, segmented neurophilic leukocytes, which also aggregated around the ductules. The portal tracts appeared to be edematous and enlarged because of the accumulation of mesenchymal cells similar to those in the lobular parenchyma, as well as because of excess ductules. The border between parenchyma and portal tracts thus appeared indistinct in hematoxylin-eosin stains whereas, in contrast, their outlines were clearly demarcated by thick connective tissue bundles in connective tissue stains. The bile ducts, portal vein, and hepatic arterial branches were unaltered, in contrast to the tributaries of the hepatic vein, the walls of which were frequently thickened and exhibited inflammatory cells occasionally pushing the endothelial lining into the lumen.

This typical picture seen in biopsy specimens appeared to be altered in a few instances by a considerably lesser degree of interstitial infiltration and spotty necrosis, by extensive necrosis of contiguous liver cells in the centrolobular zone, or by perilobular fibrosis indicating a tendency to chronicity.

In the autopsy specimens, similar spotty necrotic changes in the lobular parenchyma were frequently encountered, although they were usually associated with less inflammatory reaction. In all instances large contiguous portions of the parenchyma had become necrotic, destroying either the whole lobule (massive necrosis), the greater part of it (submassive necrosis), or at least the central and periportal zone with slitlike connections between both. In the necrotic areas the framework sometimes remained expanded by red cells and by a variable number of scavenger cells usually carrying pigment intermixed with lymphocytes and plasma cells, as well as neutrophilic and, particularly, eosinophilic segmented leukocytes (FIGURE 3). In other instances the red cells and other mesenchymal cells had disappeared to the greatest extent and the framework was collapsed, reducing the size of the lobule. The reticulum framework itself was intact and remained well separated from the thick collagenous bundles of the pre-existing portal tracts. The periphery of the "ghost lobules" was well marked by proliferating ductules, usually with irregular epithelium, frequently dilated lumens, and occasionally persisting connections with fragments of the liver cell plates (FIGURE 4). The inflammation in the smaller portal tracts was of equal and sometimes of even greater intensity than in the biopsy specimens. The larger tracts

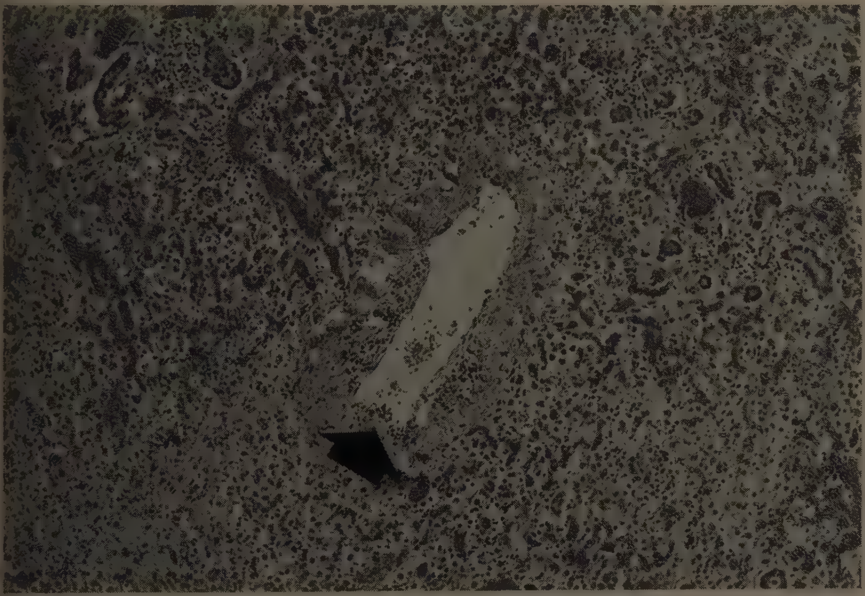

FIGURE 3. Acute massive necrotic hepatitis in autopsy specimen of patient who had received iproniazid, showing massive necrosis of liver cells being replaced by scavenger cells, proliferating ductules indicating a periportal zone, and many red cells in sinusoids. Inflammatory cells are noted in the wall of a hepatic vein tributary where they project into the lumen (*arrow*). Hematoxylin-eosin stain. ×63.

exhibited little inflammatory infiltration. The hepatic vein branches frequently showed phlebitis. The persisting portion of the parenchyma suffered rapid cellular breakdown in the acute cases. In the subacute instances cytological and architectural evidence of regeneration appeared more conspicuous and sometimes dominated the picture. The liver cells were larger than normal, and the plates became 2 cells thick and seemed to arrange themselves in a nodular fashion (FIGURE 5). Irregular areas of collapse were interspersed between the regenerating portions, particularly in the subcapsular zone. In such later stages, bile stasis was conspicuous, and the proliferated ductules were frequently filled with large and dense microcalculi, occasionally still intermixed with segmented leukocytes. In 2 instances the cytoplasm of the liver cells in contiguous portions of the parenchyma appeared rarefied and bile-pigmented, and the nuclei exhibited degenerative changes, all of which produced the picture of a bile infarct (FIGURE 6). Since in such cases regeneration seemed to have restored most of the hepatic parenchyma, which no longer showed evidence of degeneration, the fatal hepatic insufficiency was more probably related to the severe bile stasis than to morphologically recognizable damage of the liver cells.

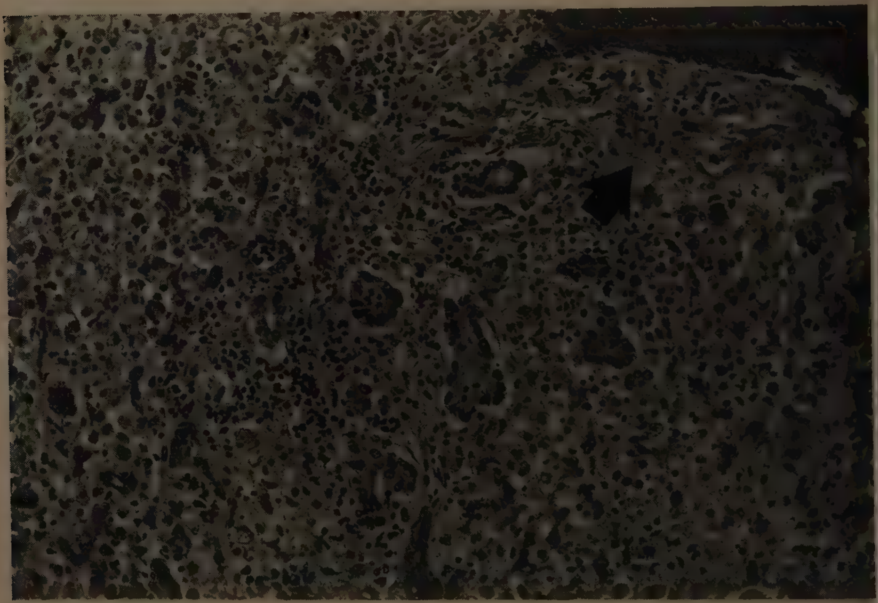

FIGURE 4. Greater magnification of FIGURE 3. Extensive proliferation of ductules with irregular epithelium and segmented leukocytes in the lumen and around them. Conspicuous cellular infiltration in and around the portal tract; the border of the latter is still recognized by thick connective tissue bundles (*arrow*). Hematoxylin-eosin stain. ×120.

Several of the histological criteria listed and found in high incidence in the material studied (TABLE 1) are typically encountered in patients who are presumed to be suffering from viral hepatitis (FIGURES 7 and 8), particularly (1) the spotty type of necrosis with replacement of single liver cells by mononu-

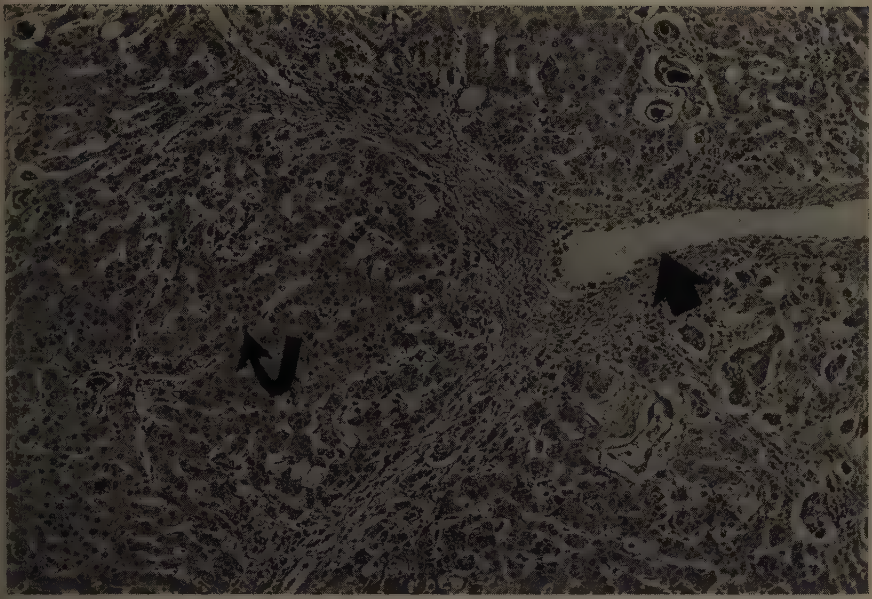

FIGURE 5. Subacute massive and submassive necrotic hepatitis in autopsy specimen of patient who had received iproniazid, showing nodular transformation of the parenchyma with several cells thick plates (*curved arrow*), ductular proliferations with severe bile stasis, and inflammatory reaction in wall of a tributary of the hepatic veins (*straight arrow*). Hematoxylin-eosin stain. ×63.

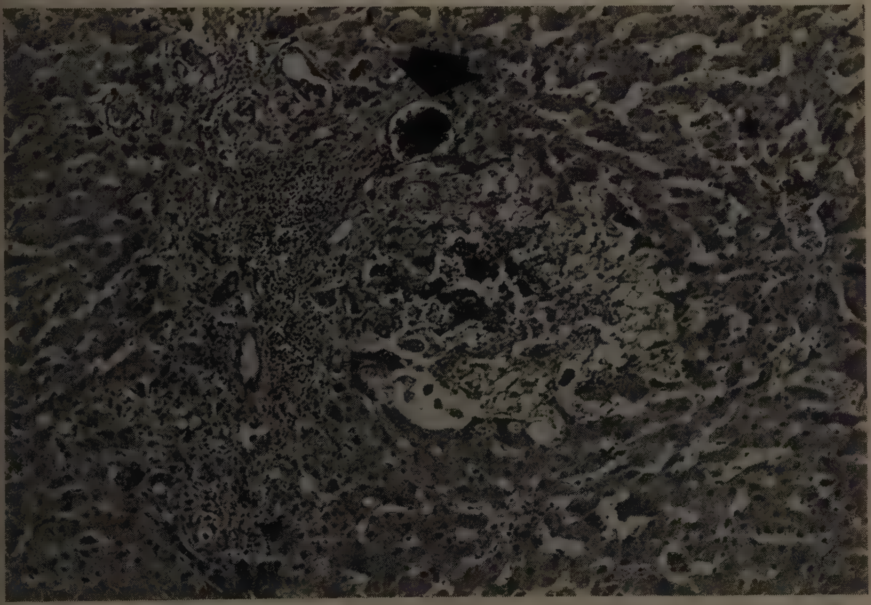

FIGURE 6. Subacute submassive necrotic hepatitis in autopsy specimen of patient who had received iproniazid, showing portal inflammatory reaction, severe bile stasis with microcalculus in ductule (*arrow*) and bile infarct. Hematoxylin-eosin stain. ×120.

TABLE 1

INCIDENCE (EXPRESSED IN NUMBER OF CASES) OF HISTOLOGICAL FEATURES IN PATIENTS WITH JAUNDICE STUDIED FOLLOWING INTAKE OF IPRONIAZID

	Specimens from			
	Biopsy		Autopsy: massive, submassive, or zonal necrotic hepatitis	
	Acute severe hepatitis	Others	Acute	Subacute or chronic
Number of cases	14	5	11	8
Spotty necrosis	14	2	4	5
Contiguous necrosis	5	0	11	7
Variation in appearance of neighboring cells	14	0	3	1
Cidophilic bodies	11	1	7	2
Lipofuscin deposition	12	2	11	5
Cytologic evidence of regeneration	14	4	4	6
Nodular regeneration	2	0	2	8
Proliferation of ductules surrounded by leukocytes	12	0	11	8
Conspicuous portal and periportal infiltration	14	4	11	8
Alterations of hepatic vein tributaries	6	0	8	4
Eosinophils	6	1	2	0
Conspicuous bile stasis	6	1	4	7
Bile infarcts	0	0	0	2

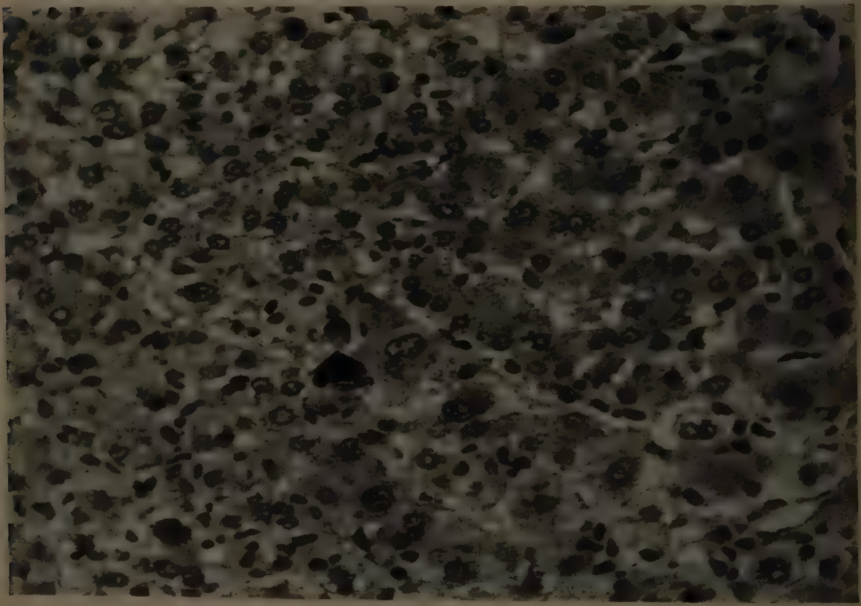

FIGURE 7. Acute spotty necrotic viral hepatitis in biopsy specimen (compare with FIGURE 1), showing great variation in the appearance of neighboring liver cells, interstitial infiltration, and acidophilic body in tissue space (*arrow*). Hematoxylin-eosin stain. ×240.

clear cells, many of them carrying pigment, (2) the acidophilic bodies, (3) the conspicuous variation in the appearance of neighboring liver cells in the nonmassively necrotic areas, (4) the ductular proliferation, (5) the portal and periportal infiltration by mesenchymal cells, (6) the conspicuous accumulation of wear-and-tear pigment, lipofuscin in Kupffer cells and portal histiocytes, and (7) the inflammatory reaction of the tributaries of the hepatic vein. None of these criteria individually permits the diagnosis of viral hepatitis. However, their combination is at present the most reliable indication of viral hepatitis[8] in view of the unavailability of specific etiological tests.

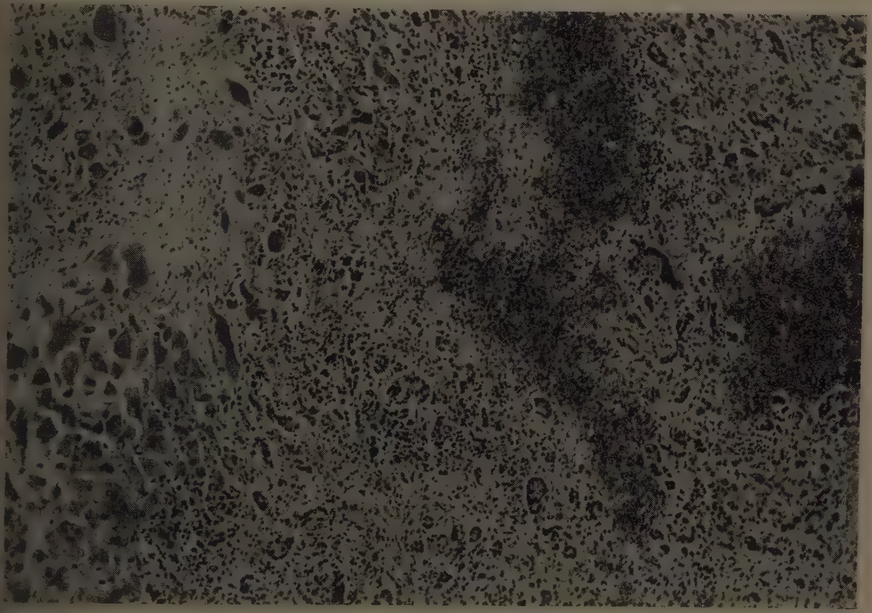

FIGURE 8. Acute massive necrotic viral hepatitis in autopsy specimen (compare with FIGURE 3), showing extensive collapse of lobules with accumulation of red cells in centrolobular zone, ductular proliferation in the periportal area, and persisting group of liver cells in left lower corner. Hematoxylin-eosin stain. ×63.

Two cases of fatal hepatic necrosis following the intake of cinchophen were taken from the files of the Mount Sinai Hospital: one showed the features of acute (FIGURE 9) and the other of subacute massive necrotic hepatitis, as described above. Through the cooperation of Frederick W. Hoffbauer, Minneapolis, Minn., sections of 2 cases who developed fatal hepatic necrosis after the intake of zoxazolamine[9] were studied, and both presented the same alterations as the cases of acute massive and submassive necrotic hepatitis following the administration of iproniazid.

Discussion

Study of additional cases confirmed the previous conclusion[7] that the histological changes in the liver in patients who developed jaundice following the intake of iproniazid represent different stages and degrees of a diffuse hepatic parenchymal disease, the picture being similar to that considered characteristic for viral hepatitis. This conclusion is based not only on the personal observation of a large number of biopsy and autopsy specimens of viral hepatitis, but even more so upon the classic descriptions of both the benign (spotty necrotic)[10-12] and fatal (massive necrotic)[13, 14] forms of viral hepatitis. No indication of a pre-existing liver disease was encountered and at least in one instance, evidence of almost complete morphologic restitution was obtained by biopsy. The main difference from the type of viral hepatitis usually seen in biopsy specimens is the higher incidence of more severe alterations in the patients who have received iproniazid. Some of the sever changes observed are otherwise rarely seen in biopsy specimens and thu provide links between the histological changes usually seen in biopsy and i autopsy specimens. Indeed, these stages provide an unusual opportunity t trace the evolution of the lesion. The relatively high number of autops specimens in the material studied does not necessarily reflect the actual mortality rate, since biopsy specimens are less easily obtained than autopsy specimens in nationwide collections. Nevertheless, the mortality rate seems to b higher in patients who develop jaundice after iproniazid intake than in vir hepatitis,[7] which is in keeping with the greater severity of the histologic changes. Both factors, however, may be the reflection of a higher-age grou in which the prognosis of hepatitis is notoriously grave, particularly in women

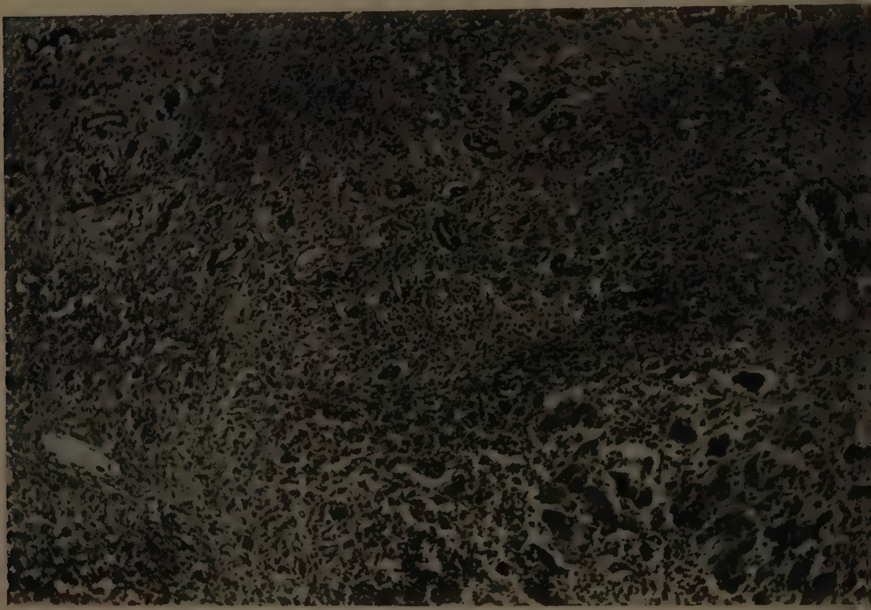

FIGURE 9. Acute massive necrotic hepatitis in autopsy specimen of patient who had received cinchophen. Persisting liver cells in right lower corner. Hematoxylin-eosin stai ×63.

A satisfactory explanation of the morphologic similarity between viral hepatitis and the jaundice following the administration of iproniazid, one that also holds true for the clinical and laboratory aspects, cannot be given at this time, particularly since the diagnosis of viral hepatitis cannot be established by etiological laboratory tests. The incidence of the jaundice excludes the assumption of a coincidental viral hepatitis quite unrelated to iproniazid therapy. The possibility must be entertained that iproniazid itself produces histological alterations that are morphologically identical with those produced by viral hepatitis. Under this assumption, the mechanism of the changes produced by a chemical in very few patients exposed to it would be the same as that caused by the virus, a hypersensitivity reaction being a possibility. The fact that other drugs of entirely different chemical nature may also produce a hepatic lesion with a very low incidence but with a high mortality rate with the same histological picture might be listed in favor of such an assumption. The changes characterized by spotty necrosis and irregular involvement throughout the liver lobule are entirely different from the common type of chemically induced hepatic injury[15] that involves contiguous zones of the lobule to the same degree and in which, therefore, a metabolic insult is better in keeping with the morphologic picture. Moreover, the types of chemically induced lesions, of which carbon tetrachloride intoxication is a good example, are easily reproduced in animals and have a clear dose relationship. The lesion following the intake of iproniazid and cinchophen is entirely different pathologically, functionally, and clinically from the cholestatic type of hepatitis occurring, apparently with a somewhat greater incidence, following the intake of various other drugs.[16]

The fact that certain drugs elicit a pathological picture resembling viral hepatitis with a very low incidence, without any dose relation and without the possibility of reproduction in animals, would indicate that the morphologic criteria for viral hepatitis are not as characteristic as has been assumed thus far. The morphologic similarity, however, may also be explained by an activation of a pre-existing subclinical viral hepatitis by iproniazid and the other drugs listed. Some of the clinical features observed as well as the relatively high severity of the pathological changes would suggest a serum (B) type of viral hepatitis. As a suggestion for further studies, the possibility must be considered that the carrier stage of serum hepatitis might occasionally be transformed by iproniazid into the active disease. Fom this viewpoint, the hepatic lesions in patients who have received iproniazid may contribute to the understanding of the still unsolved problem of the mechanism of infection of viral hepatitis.

Summary

In a study of autopsy and biopsy specimens of patients who developed jaundice following the intake of iproniazid, a diffuse parenchymal hepatic disease was observed, exhibiting all variations from spotty necrosis to massive necrosis to collapse and nodular regeneration. The histological features are identical with those considered characteristic for viral hepatitis. At this writing it is not clear whether iproniazid and other drugs produce, in a very low incidence, morphologic changes in the liver that simulate viral hepatitis

or whether a pre-existing subclinical virus hepatitis infection is transformed into a virulent disease.

Acknowledgments

I am grateful to Leo A. Pirk and Robert E. Dixon of the department of Clinical Investigation of Hoffmann-La Roche Inc., for their assistance in collecting the greater part of the material used in this study and also to the many pathologists and clinicians in all parts of the United States who assisted in this endeavor and permitted study of their material.

References

1. Scherbel, A. L. 1957. The effect of isoniazid and of iproniazid in patients with rheumatoid arthritis. Cleveland Clin. Quart. **24:** 90.
2. De Verteuil, R. L. & H. N. Lehmann. 1958. Therapeutic trial of iproniazid (Marsilid) in depressed and apathetic patients. Can. Med. Assoc. J. **78:** 131.
3. Zetzel, L. & E. Kaplan. 1958. Liver damage concurrent with iproniazid administration. New Engl. J. Med. **258:** 1209.
4. Smarr, E. R., H. Wolf & M. D. Pressman. 1958. Experience with Marsilid with report of one death. Am. J. Psychiat. **114:** 1115.
5. Frantz, A. G. 1958. Fatal jaundice associated with iproniazid (Marsilid) therapy. Report of a case. J. Am. Med. Assoc. **167:** 987.
6. Rosenblatt, M. B. 1958. Iproniazid (Marsilid) hepatitis. New York State J. Med. **58:** 3680.
7. Popper, H. 1958. Pathologic observations in jaundice associated with iproniazid therapy. J. Am. Med. Assoc. **168:** 2235.
8. Smetana, H. F., F. W. Hartman, G. A. LoGrippo, J. S. Mateer & J. Barron. 1957. Pathologic anatomy of early stages of viral hepatitis. *In* Hepatitis Frontiers, : 77. Little, Brown. Boston, Mass.
9. Hoffbauer, F. W., O. L. N. Nelson, D. J. Wagner & A. Knutsen. 1958. Fatal liver necrosis in two patients receiving zoxazolamine. Gastroenterology. **34:** 1098.
10. Axenfeld, H. & K. Brass. 1942. Klinische und bioptische Untersuchungen über den sogenannten Icterus catarrhalis. Frankfurt. Z. Pathol. **57:** 147.
11. Mallory, T. B. 1947. The pathology of epidemic hepatitis. J. Am. Med. Assoc. **134:** 655.
12. Smetana, H. F. 1954. The histopathologic diagnosis of viral hepatitis by needle biopsy. Gastroenterology. **26:** 612.
13. Lucke, B. & T. B. Mallory. 1946. The fulminant form of epidemic hepatitis. Am. J. Pathol. **22:** 867.
14. Lucke, B. 1944. Pathology of fatal epidemic hepatitis. Am. J. Pathol. **20:** 471.
15. Popper, H. & M. Franklin. 1948. Viral versus toxic hepatic necrosis. A. M. A. Arch. Pathol. **46:** 338.
16. Popper, H. & F. Schaffner. 1959. Pathology of intrahepatic cholestatic jaundice. J. Am. Med. Assoc. **169:** 1447.

Discussion of the Paper

Jesse Bollman (*Mayo Clinic, Rochester, Minn.*): Another possibility that should be considered is the increased hepatotoxicity of iproniazid (Marsilid) or of its metabolic products in the absence of hepatic function concerned with iproniazid degradation. An altered pathway of metabolism of iproniazid may produce products more toxic to the liver. In experimental animals we know that many substances show much greater toxicity after hepatectomy. In the Eck fistula of dog, carbon tetrachloride, chloroform, and other toxic agents in similar amounts produce much more hepatic necrosis than in normal animals. In dogs on a methionine-deficient diet rich in fat, the administration of alcohol in amounts that have no effect in normal animals will produce extremely fatty livers within about 2 days.

CHANGES IN THE INTRAHEPATIC CIRCULATION AND THE RETICULOENDOTHELIAL SYSTEM INDUCED BY INHIBITORS OF MONOAMINE OXIDASE*

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The chronic administration of monoamine oxidase inhibitors in man produces pathological manifestations that are believed to result primarily from their action on the liver. For the most part the toxic effects have been documented by routine pathology and clinical chemistry,[1] but to date these measurements have not been sufficiently critical to define the basic property of these drugs that is responsible for the hepatotoxic sequelae. Inasmuch as the toxic course of the syndrome had many points of resemblance to serum hepatitis, we felt that a careful study was warranted of the reticuloendothelial system (RES), which is the major defense mechanism against bacterial and viral infections. Factors that interfere with the behavior of the RES have been found, in turn, to lead to damage of the parenchymal elements of the liver. Impairment of the RES was associated with a greatly increased susceptibility of the animal to infectious processes and to various types of systemic and local injury. Study of the RES has been greatly facilitated by the recent development of methods for quantitatively measuring the phagocytic function of the RES and, by this means, for measuring liver blood flow.[2]

In essence, a colloidal suspension of carbon is injected into the blood stream and the rate of clearance of the colloid is then studied. The circulating carbon is removed from the liver proper by the phagocytic activity of the Kupffer elements. Under normal circumstances the topographical distribution of carbon within the liver lobule reflects the pattern of blood flow through the tissue, the uptake of carbon by the Kupffer cells being a function of the concentration in the blood stream and the volume of blood flowing past the RE elements. Histological inspection shows that the phagocytized material is scattered diffusely throughout the lobule, with the Kupffer cells in the periportal region showing a somewhat heavier coloration. Under abnormal circumstances, the uniform pattern is no longer present. The absence of carbon in particular areas can be attributed to one of two possible causes, namely, interference with the phagocytic mechanism per se, or to a curtailment of the circulation through these sinusoids. By measuring both the rate of clearance as an index of the phagocytic activity and the histological distribution of carbon it is possible to obtain a broad over-all approximation of liver circulation.

The rate at which carbon is cleared from the blood stream has been used as a quantitative measure of the phagocytic function of the RES.[2] In the case of colloidal carbon more than 80 per cent of the carbon is taken up by the liver elements and less than 10 per cent by the spleen, the other major tissue containing large numbers of phagocytic elements. The carbon-clearance technique, therefore, measures essentially the phagocytic function of the RES in the liver.

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* The work reported in this paper was supported in part by a grant from Hoffmann-La Roche Inc., Nutley, N. J.

† Established Investigator of the American Heart Association, New York, N. Y.

As has been shown,[3] the phagocytic activity of the RES can be increased or decreased by appropriate stimuli. Repeated exposures to denatured proteins or to polysaccharide complexes such as zymosan have been found to lead to a more efficient clearance of colloidal carbon. Under such circumstances the over-all activity of the Kupffer elements is increased so that, on histological inspection, the liver appears uniformly dark. On the other hand, the functional activity of the RES can be suppressed by factors such as infection. In these animals the liver appears uniformly lighter, and the carbon is distributed in a fine, dustlike manner throughout the tissue.

These two considerations indicate that, under different experimental conditions, we may be faced with various combinations of changes in blood flow and/or changes in the capacity of the cells to remove carbon from the bloodstream. Our interpretation of the findings must take into account the several possibilities in this regard.

Table 1
Monoamine Oxidase Inhibitors

Marsilid*	1-Isopropyl-2-isonicotinylhydrazine
JB-516	Phenyl-isopropylhydrazine
RO 5-0994*	1,2,3,4-Tetrahydrophthalazine HCl
RO 2-6797*	2-Ethyl-benz-f-isoindoline citrate
RO 5-0700*	2-Benzyl-1-picolinyl hydrazine
IPH†	Isopropylhydrazine

* Hoffmann-La Roche.
† Isopropylhydrazine.

The present studies were carried out in CFN-strain rats and albino rabbits. The greater part of the investigation was concerned with the effects of iproniazid (Marsilid*) and JB-516.† Representative experiments were also run on a series of newly developed hydrazines that are potent monoamine oxidase inhibitors (TABLE 1). In the acute experiments the animals were given the drugs in selected dose ranges, and measurements were recorded 1 to 4 hours after administration. Experiments were also carried out in which the anima received 2 large doses of the drug on consecutive days. Chronic administration of the drug was followed in both normotensive and hypertensive rats over a period of 5 weeks.

Results

Altered liver circulation following monoamine oxidase inhibitors. In the acut experiments, rats and rabbits were given 1 to 50 mg./kg. of Marsilid. It was felt that the resulting inhibition of monoamine oxidase and the subsequen interference with the degradation of vasoactive amines might be reflected by a change in the pattern of distribution of blood through the liver. Carbon (4 mg./150-gm. rat) was injected intravenously several hours after Marsilid administration, and the livers were then prepared by frozen section for microscopic inspection. Liver sections of Marsilid-treated animals showed a characteristic alteration in the distribution of carbon. In contrast to controls, the carbon was restricted to the Kupffer cells along the edges of the lobule, the central portion of each unit being almost completely devoid of carbon (FIGURES 1 and 2). The distorted pattern began to develop within 2 to 4 hours after the drug was administered, but became most pronounced after the second day of treatment. With doses below 5 mg./100 gm. of Marsilid

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* Hoffmann-La Roche.
† Lakeside.

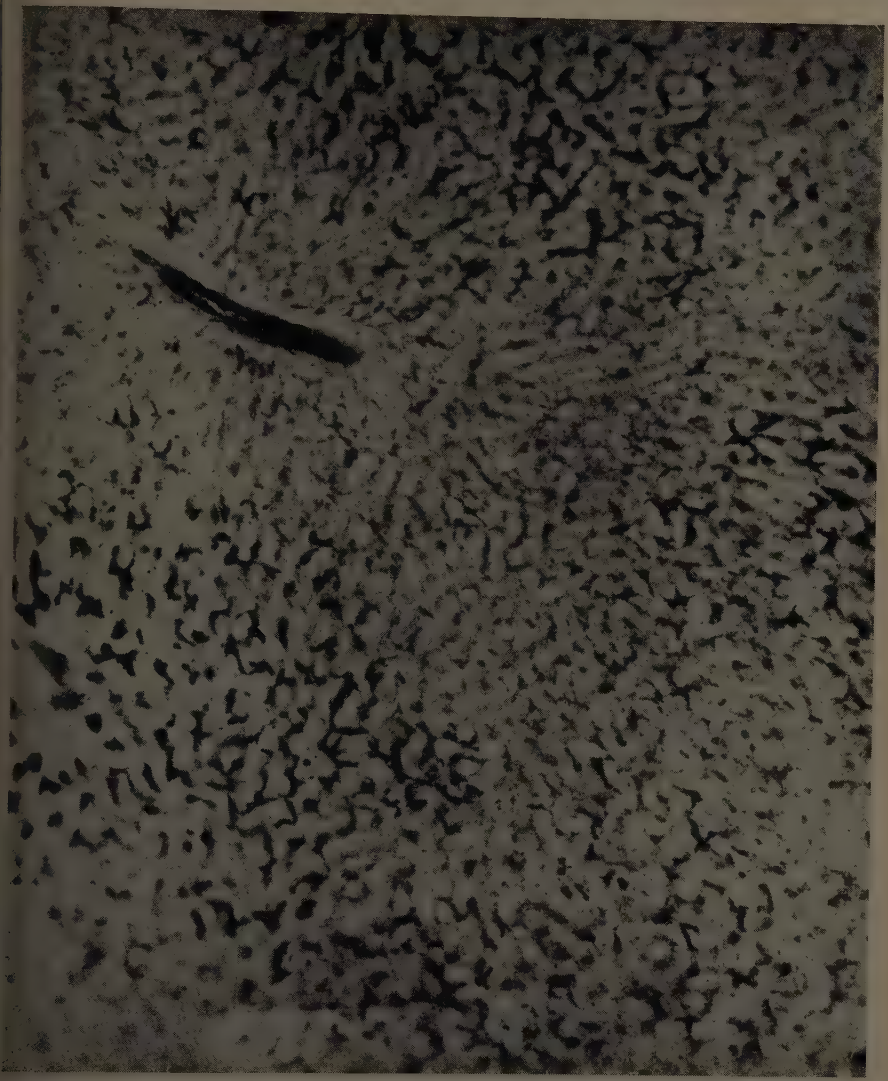

FIGURE 1. Frozen section of liver of control rat that had received intravenous injection of 4 mg. carbon/100 gm. body weight. Note the uniform deposition of carbon in the Kupffer cells throughout the lobule. ×35.

the vascular effect was much less consistent and not as severe. Although individual variations were encountered in the severity of the effect, all the treated animals showed an atypical distribution of carbon.

Phagocytic function of the reticuloendothelial system. What, then, is the significance of the aberrant pattern of carbon deposition? The findings resemble the vascular changes encountered in animals subjected to traumatic

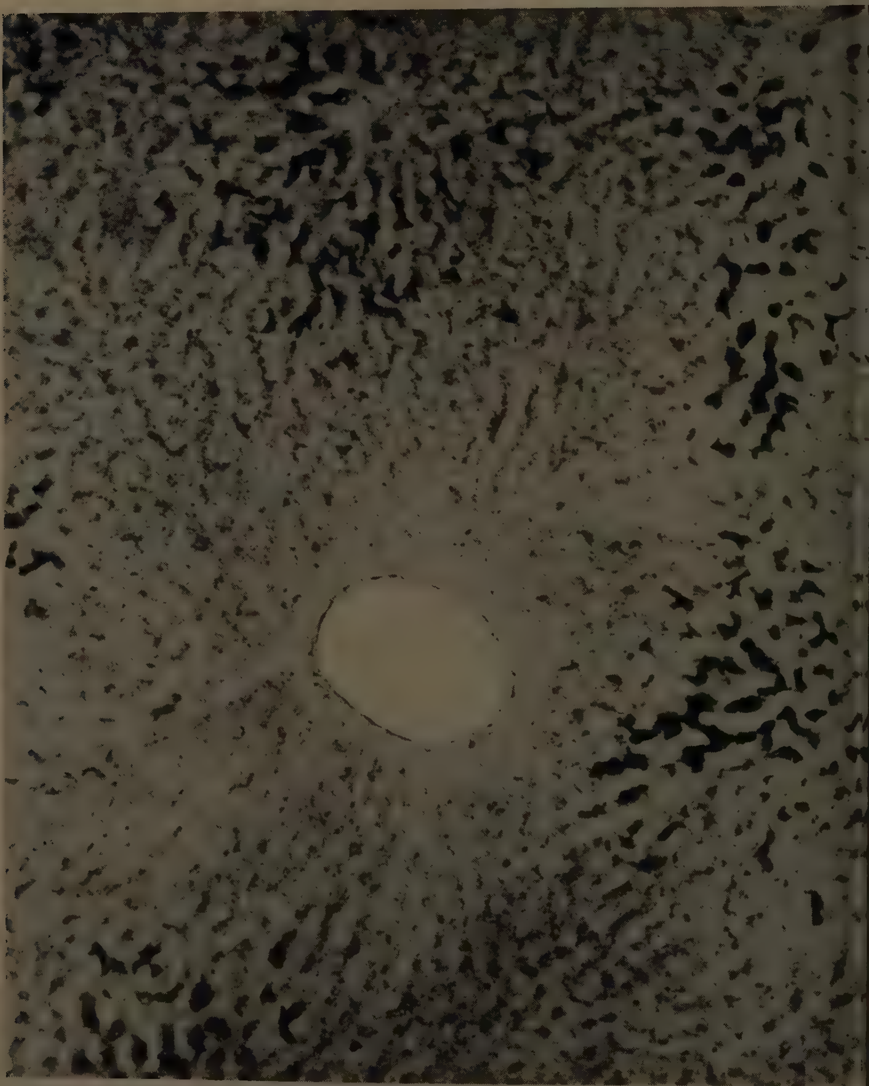

FIGURE 2. Frozen section of liver from rat that had received intravenous injection of mg. carbon/100 gm. body weight and that had also been injected intraperitoneally with mg. Marsilid/150 gm. body weight for 2 days. Note the absence of carbon in the sinusoi around the central vein. The Kupffer cells in the periportal zone continue to show carbo ×35.

or hemorrhagic shock. In the latter experiments the abnormal distribution of carbon was found to be due to a restricted blood flow through the liver. It was therefore necessary to determine whether the phagocytic properties of the reticuloendothelial cells in the center of the lobule had been selectively depressed by the monoamine oxidase inhibitor or whether the intrinsic circulation through the liver lobule had been altered.

Studies were therefore carried out in which the removal of carbon from the blood stream was measured quantitatively by a technique developed by Benacerraf and his co-workers.[2] The rate of clearance of inert colloidal particles from the blood stream is followed in specimens of blood removed at 2 to 3 minute intervals for about 20 to 30 minutes, and the concentration of carbon is determined by colorimetry. Similar studies have been carried out using radioactive albumin or chromium phosphate.[5] In the present study, carbon was used, since it can be measured colorimetrically, and the same material can be subsequently examined histologically to study the pattern of distribution of the colloid.

Table 2

Effect of MAO Inhibitors on Reticuloendothelial System

Agent	Dose mg./kg.	Phagocytic index K—Carbon*
Controls—saline	—	.01 ± .004
Marsilid	10–100	.01
JB-516	20	.011
RO 5-0994	20	.009
RO 2-6797	20	.012
IPH	20	.008

* Measured 1 hour after drug had been administered intraperitoneally.

As shown in TABLE 2, pretreatment with Marsilid had no significant effect on the rate at which carbon was cleared by the RES. Hence, the reduced deposition of carbon in the central lobular region could not be ascribed to a depressed function of the RES elements. The only alternative explanation was that the blood flow through the liver had been reduced in the central lobular region, whereas the amount of blood passing through the periportal areas had been commensurately increased.

Why, then, was there a redistribution of intrahepatic blood flow following treatment of the animal with monoamine oxidase inhibitors? It was felt that, if the phenomenon was due to an inhibition of monoamine oxidase per se, the same effect should be evident in animals treated with other inhibitors of this category. Furthermore, the severity of the changes should be related to the relative effectiveness of different agents as inhibitors. Studies were therefore conducted with 5 other known inhibitors of monoamine oxidase. Each of these agents produced a comparable change in the pattern of carbon deposition, presumably as a consequence of a shift in the pattern of blood flow through the liver (TABLE 3). This effect was not obtained with isoniazid (1 to 10 mg./150 gm.), which is not a monoamine oxidase inhibitor. However, those inhibitors that were presumably more potent in terms of their effect on brain tissue homogenates studied *in vitro* did not have a correspondingl greater effect on the pattern of blood flow. It is of some interest that a sim lar distortion in carbon deposition could be induced by MAO inhibitors in an mals pretreated with zymosan so as to stimulate RE activity.

Inasmuch as a number of studies have indicated that isopropylhydrazin (IPH), a breakdown product of Marsilid, might represent the toxic compo nent associated with liver damage, studies were also carried out with th agent (10 mg./kg.). Repeated doses of IPH given over a period of severa days produced a curious phenomenon: histological studies revealed that bloo was being dammed up in the sinusoids, particularly toward the tributaries the portal vein. The sinusoids draining into the hepatic vein appeared norma and usually devoid of blood. Subsequently, a slight change in carbon depos tion became apparent, although the over-all effect was not as marked as wit the monoamine oxidase inhibitors.

Deleterious effects of Marsilid following blockade of the RES. As indicate above, monoamine oxidase inhibitors have no direct effect on the phagocyt function of the RES. Under various experimental circumstances, particular infections, endotoxemia, and protracted hypotension, the RES becomes d pressed; that is, its capacity to clear colloids from the blood stream is signi cantly impaired. Furthermore, under such circumstances, in contrast to th situation in normal animals, it is difficult or impossible to stimulate the RE The possibility was therefore entertained that, in instances where Marsil was superimposed on an underlying derangement of the RES, the drug mig serve to intensify this defect and possibly limit the capacity of the system respond to stimuli.

TABLE 3

EFFECT OF MONOAMINE OXIDASE INHIBITORS ON INTRAHEPATIC DEPOSITION OF CARB

Agent	Dose (mg./100 gm.)	Distribution of carbon
Controls	—	Diffuse
Marsilid	5–10	Restricted
JB-516	5	Restricted
RO 5-0994	5–10	Diffuse
RO 5-0700	5	Restricted
IPH	4–10	Diffuse

This contingency was tested by administering Marsilid to animals in whic the phagocytic function of the RES had been depressed by pretreating the with bacterial endotoxins or by the administration of a colloid in sufficie amount to bring about blockade of the RES. Two effects were demonstrate Following RES blockade in the rat, Marsilid further depressed the phagocyt activity of these cells. In fact, many animals subjected to a regimen of co bined RES blockade and Marsilid became quite ill and, on histological inspe tion, showed evidence of focal necrosis of the liver. A second effect encoun tered with this combined treatment was a significant lag in the return of th RES to normal activity levels. Under normal circumstances, blockade of th RES with carbon is followed by recovery within 8 to 12 hours, and there an actual enhancement of phagocytic activity for several days thereafter. Blockaded animals that had received Marsilid continued to show a depressed phagocytic function for 4 or 5 days.

Increased susceptibility to shock following Marsilid. The fact that Marsilid interfered with the activity of the RES when this system was subnormal led us to investigate the effect of these inhibitors on the reaction to stress itself. In the present experiments rats were exposed to a standard form of traumatic shock induced by rotating the animals in the Noble-Collip drum, as well as to a form of hemorrhagic shock induced by protracted hypotension. In both instances Marsilid increased the lethal outcome of the shock procedure (TABLE 4). For example, rats receiving a normally well-tolerated episode of drum shock, after treatment with 10 mg./kg. of Marsilid showed an $LD_{75 to 80}$; in controls, 650 turns under identical conditions result in an $LD_{30 to 40}$.

These findings may be relevant to the hepatotoxic potentiality of Marsilid, since the shock syndrome is associated with extensive liver damage and a systemic toxemia. In this regard, mention should be made of experiments in which Marsilid-treated rats and rabbits received single large doses of an *Escherichia coli* endotoxin intraperitoneally. No unusual toxicity was encountered under these circumstances. The latter finding should be compared with the data cited above in which RES blockade, followed by Marsilid, was definitely more toxic. Evidently, some form of cellular damage predisposes the liver to the subsequent potentiality of Marsilid to interfere further with cellular function.

TABLE 4
PREDISPOSING ACTION OF MONOAMINE OXIDASE INHIBITORS ON SHOCK

Agent	Dose (mg./kg.)	Survival*
Controls	—	14/20 (70 per cent)
Marsilid	10	10/20 (50 per cent)
Marsilid	20	3/20 (15 per cent)
JB-516	10	4/20 (20 per cent)
IPH	10	12/20 (60 per cent)

* Rats exposed to 650 turns in Noble-Collip drum.

Effects on microcirculation. It seemed reasonable to assume that monoamine oxidase inhibitors should affect vascular reactivity by interfering with the handling of vasoactive amines such as adrenaline, noradrenaline or 5-hydroxytryptamine. For this purpose the microcirculation of the mesentery and skin was visualized, and the vasoconstrictor response to catecholamines was used as an index of the reactivity of the small blood vessels.[6] Vascular responses to adrenaline or noradrenaline were not enhanced in Marsilid-treated rats, and, in many animals, such responses were actually depressed. The intravenous administration of Marsilid was followed by dilation of the arterioles and precapillaries and an over-all increase of blood flow through the capillary bed. The vascular response to histamine and acetylcholine was not affected by Marsilid, both of these agents giving a normal pattern of reaction in drug-treated animals.

The possibility that Marsilid might predispose the small blood vessels to the effects of local tissue injury was also examined. This contingency was tested in the rat and rabbit mesentery by local microtrauma and, in the skin of the rabbit, by noting the evolution of the dermal necrosis characteristic of the local Shwartzman reaction. In neither case did Marsilid produce an increased tendency to tissue damage.

Summary

The evidence presented suggests that monoamine oxidase inhibitors alter the intrahepatic distribution of blood, chiefly in the direction of a diminished blood flow through the central portion of the lobule. In addition, Marsilid has a clear-cut predisposing action in furthering the toxic effects associated with blockade of the reticuloendothelial system. It is possible that the hepatotoxic action of this drug may be related to its effect on this system, particularly in view of the importance of the RES as a defense mechanism against bacterial and viral infections. The effects produced by isopropylhydrazine suggest that monoamine oxidase inhibitors, in addition to their demonstrated effect on the destruction of catecholamines, may have a direct metabolic effect.

References

1. Popper, H. 1958. Pathological findings in jaundice associated with iproniazid therapy. J. Am. Med. Assoc. **168:** 2235.
2. Biozzi, G., B. Benacerraf & B. N. Halpern. 1953. A study of the kinetics of the granulopectic activity of the RES in relation to the dose of carbon injected. Brit. J. Exptl. Pathol. **34:** 441.
3. Biozzi, G., B. N. Halpern, B. Benacerraf & C. Stiffel. 1957. Phagocytic activity of the reticulo-endothelial system in experimental infections. *In* Physiopathology of the Reticulo-Endothelial System. B. N. Halpern, Ed. Thomas. Springfield, Ill.
4. Zweifach, B. W. & B. Benacerraf. 1958. Effect of hemorrhagic shock on the phagocytic function of Kupffer cells. Circulation Research. **6:** 83.
5. Dobson, E. L. & M. B. Jones. 1952. The behavior of intravenously injected particulate material. Acta Med. Scand. **144:** Suppl. 273.
6. Zweifach, B. W. & D. B. Metz. 1955. Relation of blood-borne agents acting on mesenteric vascular bed to general circulatory reactions. J. Clin. Invest. **34:** 653.

Discussion of the Paper

Jesse Bollman (*Mayo Clinic, Rochester, Minn.*): Zweifach has shown changes in the circulation of liver and the clearance of colloidal particles after the administration of iproniazid (Marsilid). In the light of our present knowledge of circulation of the liver, as well as the functions of the reticuloendothelial system, interpretations of these phenomena remain in the realm of speculation. Zweifach's report should promote interest in the study of these fields. Certainly, these interesting phases of hepatic circulation in reticuloendothelial function offer promising fields for investigation.

CLINICAL TOXICITY STUDIES OF A MONOAMINE OXIDASE INHIBITOR

I. J. Greenblatt and A. Kahn
Beth-El Hospital, Brooklyn, N. Y.

Zeller[1] suggested the name monoamine oxidase for an enzyme (described by Hare,[2] Pugh and Quastel,[3] and others) that is capable of oxidizing amines *in vivo*. He wished to prevent confusion with the diamine oxidases. The enzyme is widely distributed in animal tissues, and has been adequately reviewed by Davison.[4]

Iproniazid (Marsilid*) inhibits the oxidase; isoniazid is without effect. More recently, phenylisopropylhydrazine (JB-516, or Catron†) has been demonstrated as a highly potent monoamine oxidase inhibitor.[5] Iproniazid and JB-516 have received extensive clinical trials in treatment of such disorders as hypertension, mental depressive states, and angina pectoris. Because of the obviously wide clinical application of these drugs, chronic toxicity studies have been undertaken. The particularly undesirable effects of a viral type of hepatitis encountered during iproniazid therapy have led to limitation in use of the drug in many clinical areas. The highly potent JB-516, the basis for our study, is about 8 times as effective in humans as iproniazid.

TABLE 1
HUMAN SEMEN STUDIES*

Subject	Control		1 Month		2 Months	
	Millions/cc.	Motility (percentages)	Millions/cc	Motility (percentages)	Millions/cc	Motility (percentages)
S.H.	96.5	80+	104.1	80+	88.1	80+
B.L.	104	80+	83.2	80+	95.8	80+
E.S.	87.1	90+	96.0	80+	84.0	80+
P.E.	113	80+	105.5	80+	100.4	80+

* Dosage: 6.25 mg. JB-516 per day, orally.

Toxicity Studies

Semen studies. Because of the widespread pharmacological effects of the drug, 4 normal adults were selected for these studies. They received 6.25 mg. of the drug, by mouth, for 2 months. They did not report any decrease in libido, or any evidence of impotence during this period. Their semen counts and sperm activity remained essentially unchanged (TABLE 1).

Urinary bladder. Five subjects were used in studies of effects of the drug on the innervation of the urinary bladder as measured by cystometric changes. Increments of water at approximately 50 cc. per unit time were instilled. In

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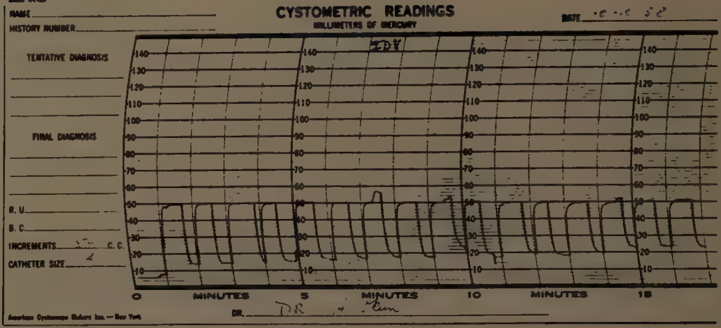
* Hoffmann-La Roche.
† Lakeside.

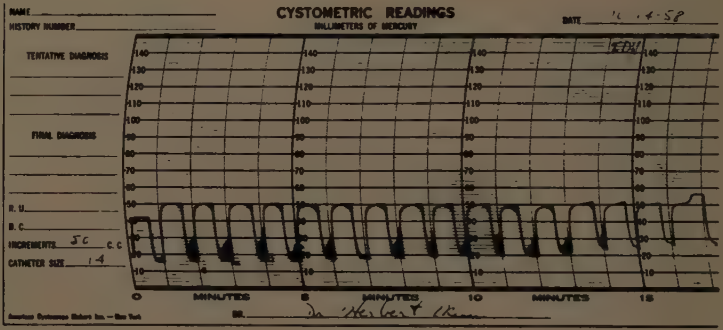
BEFORE



CYSTOMETRIC READINGS
MILLIMETERS OF MERCURY

NAME
HISTORY NUMBER
DATE [illegible]
TENTATIVE DIAGNOSIS
FINAL DIAGNOSIS
R. U.
B. C.
INCREMENTS [illegible] C. C.
CATHETER SIZE 4
DR. DR [illegible] Klein
American Cystoscope Makers Inc. — New York

AFTER



CYSTOMETRIC READINGS
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NAME
HISTORY NUMBER
DATE 10-4-58
TENTATIVE DIAGNOSIS
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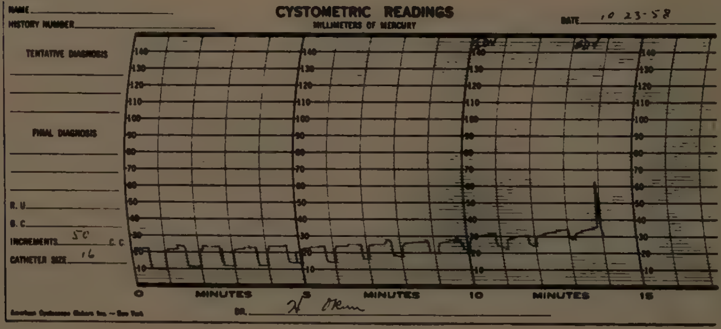
R. U.
B. C.
INCREMENTS 50 C. C.
CATHETER SIZE 14
DR. Dr. Herbert Klein
American Cystoscope Makers Inc. — New York

BEFORE



CYSTOMETRIC READINGS
MILLIMETERS OF MERCURY

NAME
HISTORY NUMBER
DATE 10-23-58
TENTATIVE DIAGNOSIS
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R. U.
B. C.
INCREMENTS 50 C. C.
CATHETER SIZE 16
DR. H Klein
American Cystoscope Makers Inc. — New York

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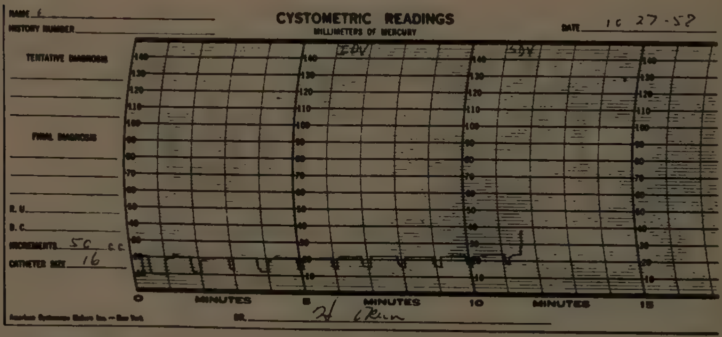
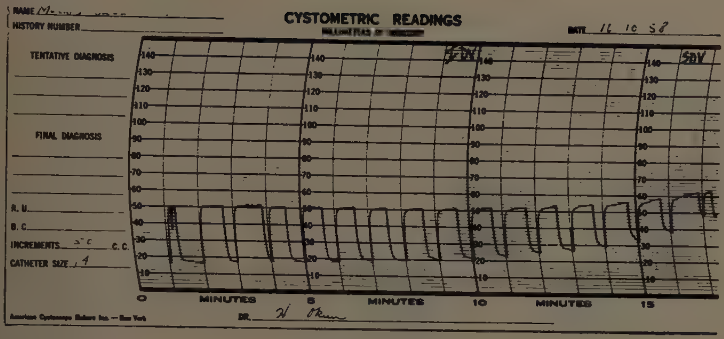


CYSTOMETRIC READINGS
MILLIMETERS OF MERCURY

NAME L
HISTORY NUMBER
DATE 10-27-58
TENTATIVE DIAGNOSIS
FINAL DIAGNOSIS
R. U.
B. C.
INCREMENTS 50 C. C.
CATHETER SIZE 16
DR. H Klein
American Cystoscope Makers Inc. — New York

FIGURE 1. Effects on the urinary bladder of JB-516 at a dosage of 100 mg./day for 4 day

BEFORE



NAME M.

HISTORY NUMBER

CYSTOMETRIC READINGS

MILLIMETERS OF MERCURY

DATE 10 10 58

TENTATIVE DIAGNOSIS

FINAL DIAGNOSIS

R. U.

B. C.

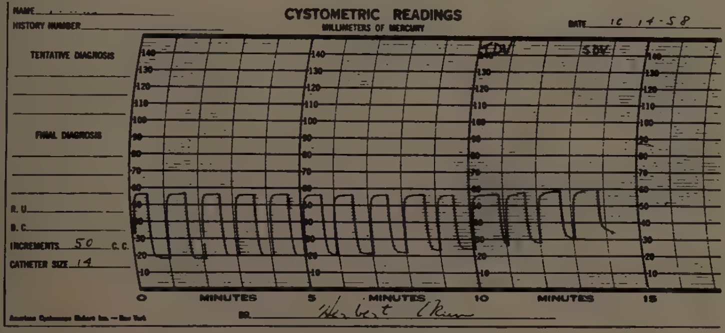
INCREMENTS 50 C.C.

CATHETER SIZE 14

DR. H Oken

American Cystoscope Makers Inc. — New York

AFTER



NAME

HISTORY NUMBER

CYSTOMETRIC READINGS

MILLIMETERS OF MERCURY

DATE 10 14-58

TENTATIVE DIAGNOSIS

FINAL DIAGNOSIS

R. U.

B. C.

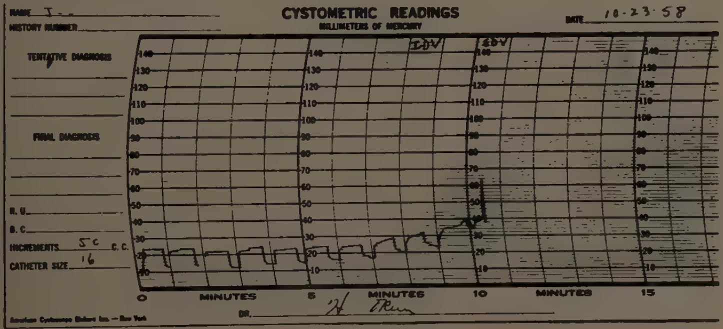
INCREMENTS 50 C.C.

CATHETER SIZE 14

DR. Herbert Oken

American Cystoscope Makers Inc. — New York

BEFORE



NAME J

HISTORY NUMBER

CYSTOMETRIC READINGS

MILLIMETERS OF MERCURY

DATE 10-23-58

TENTATIVE DIAGNOSIS

FINAL DIAGNOSIS

R. U.

B. C.

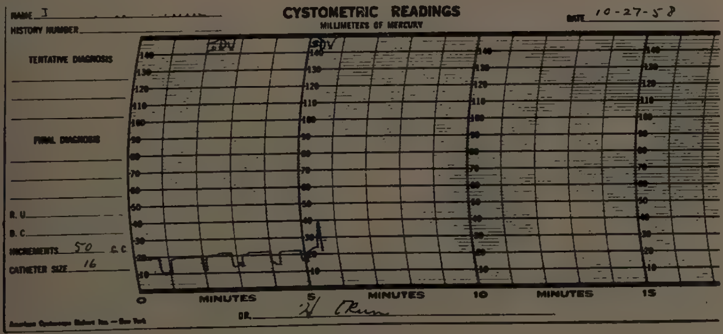
INCREMENTS 50 C.C.

CATHETER SIZE 16

DR. H Oken

American Cystoscope Makers Inc. — New York

AFTER



NAME J

HISTORY NUMBER

CYSTOMETRIC READINGS

MILLIMETERS OF MERCURY

DATE 10-27-58

TENTATIVE DIAGNOSIS

FINAL DIAGNOSIS

R. U.

B. C.

INCREMENTS 50 C.C.

CATHETER SIZE 16

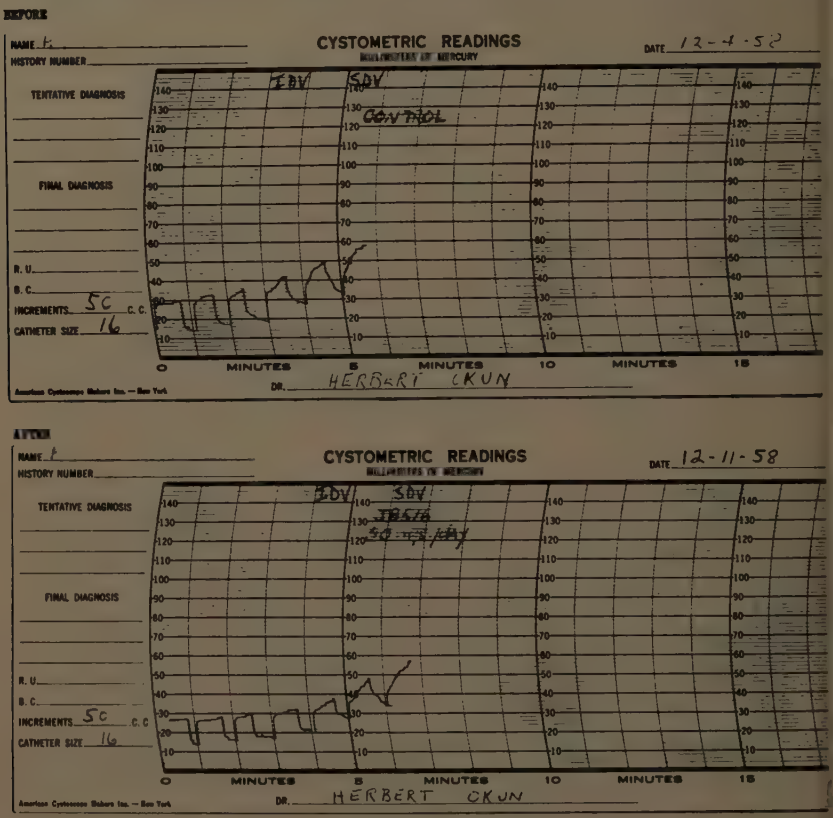
DR. H Oken

American Cystoscope Makers Inc. — New York

FIGURE 2. Effects on the urinary bladder of JB-516 at a dosage of 100 mg./day for 4 days.

all 4 male subjects, no essential changes were observed between the contr reading and after the ingestion of 100 mg. of JB-516 for 4 consecutive days In the fifth subject, 50 mg. per day for 7 days produced only a questionab difference (FIGURES 1, 2, and 3).

Urine 17-keto and 17-OH steroids. Three subjects were placed on 25 mg of the drug per day for 4 weeks; 1 received the drug for 2 weeks. The urin



CYSTOMETRIC READINGS
MILLIMETERS OF MERCURY
NAME L
HISTORY NUMBER
DATE 12-4-58
TENTATIVE DIAGNOSIS
FINAL DIAGNOSIS
R. U.
B. C.
INCREMENTS 50 C. C.
CATHETER SIZE 16
DR. HERBERT CKUN

CYSTOMETRIC READINGS
MILLIMETERS OF MERCURY
NAME L
HISTORY NUMBER
DATE 12-11-58
TENTATIVE DIAGNOSIS
FINAL DIAGNOSIS
R. U.
B. C.
INCREMENTS 50 C. C
CATHETER SIZE 16
DR. HERBERT CKUN

FIGURE 3. Effects on the urinary bladder of JB-516 at a dosage of 50 mg./day for 7 day

steroids were determined by conventional techniques; the urines in TABLE represent 24-hour samples. Subject L.G. showed a significant rise in th 17-ketosteroids, as did R.G. The latter showed an unusual increase in th 17-OH values after 2 weeks. J.T. was an aged cachexic individual.

Serum electrophoresis. Eleven subjects under therapy with JB-516 for 1 to 41 weeks showed no significant changes (N.S.C.) except in the alpha- globulins. Patients R.D. and J.W. showed an increase in alpha-1 globuli of 11 and 13 per cent, respectively (TABLE 3). The method used paper strip with a Spinco Duostat and Analytrol.

Renal functions. Periodic spot checking of urines for specific gravity, suga albumin, and microscopic matter showed no significant changes from the control urine on 7 subjects receiving 6.25 mg. of the drug per day for 2 months. Four subjects had P.S.P. tests performed after ingestion of 12.5 mg. per day of the enzyme inhibitor for 7 to 11 days (TABLE 4). No changes of clinical significance were observed.

TABLE 2
URINE 17-KETO AND 17-OH STEROIDS

Subject	Control		2 Weeks		4 Weeks	
	17K	17-OH	17K	17-OH	17K	17-OH
L.G.	11.4	2.0	14.9	2.3	17.1	3.6
H.C.	8.3	6.6	6.4	6.1	8.8	4.9
R.G.	10.9	3.8	19.2	12.5		
J.T.	3.1	2.8	2.8	2.0	3.4	2.2

* Dosage: 25 mg. JB-516 per day, orally.

TABLE 3
BLOOD SERUM ELECTROPHORESES*

Subject	Duration of therapy (weeks)	Albumin	a1	a2	b	*a*
R.K.	12	N.S.C.	N.S.C.	N.S.C.	N.S.C.	N.S.C.
M.M.	19	N.S.C.	N.S.C.	N.S.C.	N.S.C.	N.S.C.
M.T.	11	N.S.C.	N.S.C.	N.S.C.	N.S.C.	N.S.C.
H.S.	19	N.S.C.	N.S.C.	N.S.C.	N.S.C.	N.S.C.
I.L.	41	N.S.C.	N.S.C.	N.S.C.	N.S.C.	N.S.C.
M.P.	33	N.S.C.	N.S.C.	N.S.C.	N.S.C.	N.S.C.
R.D.	12	N.S.C.	Increase 11%	N.S.C.	N.S.C.	N.S.C.
J.W.	16	N.S.C.	Increase 13%	N.S.C.	N.S.C.	N.S.C.
L.L.	10	N.S.C.	N.S.C.	N.S.C.	N.S.C.	N.S.C.
J.M.	24	N.S.C.	N.S.C.	N.S.C.	N.S.C.	N.S.C.
I.M.	20	N.S.C.	N.S.C.	N.S.C.	N.S.C.	N.S.C.

* Dosage: 6.25 mg. JB-516 per day, orally.

TABLE 4
P.S.P. TESTS FOR RENAL FUNCTION*

	Before (percentages)	No. of days administered	After (percentages)
M.S. #52701	74	10	79
M.C. #3A	68	10	63
J.B. #52535	64	11	66
S.S. #3A	70	7	73

* Dosage: 12.5 mg. JB-516 per day, orally.

Hemograms. Twenty-two patients, on 6.25 mg. of oral JB-516 for 6 to 35 weeks showed minimal, if any, significant changes in hemoglobin, white blood cell counts, differential, or hematocrits. One subject showed a drop in platelets and 1 a moderate increase in prothrombin time (TABLE 5).

Thyroid functions. Serum protein–bound iodine assays showed an increase beyond the normal values (up to 8 μg./100 ml.) in 2 of 6 subjects who were administered the drug for 8 to 13 weeks. Although neither value was unusually high, the general tendency was for the serum levels to increase after th onset of therapy at this level (TABLE 6).

TABLE 5
HEMOGRAMS*

No. of subjects	Duration of therapy (weeks)	Hb	WBC	Platelets	Differential
7†	6–10	N.S.C.	N.S.C.	N.S.C.	N.S.C.
6‡	11–15	N.S.C.	N.S.C.	N.S.C.	N.S.C.
6	21–25	N.S.C.	N.S.C.	N.S.C.	N.S.C.
3	31–35	N.S.C.	N.S.C.	N.S.C.	N.S.C.

There were no significant changes in hematocrits; prothrombin times of patients on 8 to 10 weeks of therapy showed no changes.

* Dosage: 6.25 mg. JB-516 per day, orally.

† One patient showed increased prothrombin from 14 to 19 sec.

‡ One patient showed a drop in the number of platelets from 360,000 to 110,000.

TABLE 6
SERUM PROTEIN–BOUND IODINE*

Subject	Duration of therapy (weeks)	Before (μg./100 ml.)	After (μg./100 ml.)
D.R.E.	9	4.8	5.7
D.R.S.	9	6.9	7.4
R.K.	13	7.3	6.2
M.T.	11	7.1	7.0
P.D.	14	6.4	8.4
A.R.	8	6.6	8.9

* Dosage: 6.25 mg. JB-516 per day, orally.

TABLE 7
GLUCOSE-TOLERANCE CURVES*

Subject	Before					After 1 month				
	Fast	½ hr.	1 hr.	2 hr.	3 hr.	Fast	½ hr.	1 hr.	2 hr.	3 hr.
S.H.	91	103	134	100	70	72	100	124	81	66
E.S.	82	118	150	73	83	100	114	130	90	78
B.L.	90	120	129	91	92	84	106	149	96	97
P.E.	74	100	138	84	110	83	102	140	91	75
J.T.	184	281	297	303	307	206	255	260	271	288
H.T.	83	92	130	101	88	71	100	104	99	63

* Dosage: 12.5 mg. JB-516 per day, orally.

Glucose-tolerance tests. After 1 month of study on 6 subjects, of whom was a diabetic, no clinically significant changes were observed, although 5 the 6 showed a slight decrease of the postdrug ingestion level compared wit

TABLE 8
GLUCOSE-TOLERANCE CURVES

Subject	Before					2 Months after				
	Fast	½ hr.	1 hr.	2 hr.	3 hr.	Fast	½ hr.	1 hr.	2 hr.	3 hr.
S.H.	91	103	134	100	70	76	88	91	70	64
E.S.	82	118	150	73	83	71	82	88	66	59
H.T.	83	92	130	101	88	66	80	81	58	54

* Dosage: 12.5 mg. JB 516 per day, orally.

TABLE 9
RESULTS OF ATTEMPTED INHIBITION OF H.I.A.A. IN THE URINE* WITH JB-516 AND MARSILID

Case: L. M. (malignant carcinoid)

Drug	Dosage	Response
Marsilid:	400 mg./day, orally (3 days)	No inhibitions of H.I.A.A.
JB-516:	100 mg./day, orally (3 days)	Definite, but *partial* inhibition

* Diluted 1:7.

TABLE 10
LIVER PROFILES*

No. of subjects	Duration of therapy (weeks)	Serum bilirubin	Cephalin flocculation	Thymol turbidity	SGOT	A/G	Net change
6	8 to 10	Normal	Normal	Normal	Normal	Normal	0
21	10 to 20	Normal	Normal	Normal	Normal	Normal	0
1	21 to 25	Normal	Normal	Normal	Rise	Normal	Cephalin + to ++ SGOT 29 to 58
1	21 to 25	Normal	Normal	Normal	Rise	Normal	SGOT 37 to 61
1	21 to 25	Normal	Normal	Rise	Rise	Change	T.T. 4.1 to 5.2 SGOT 21 to 46
3	26 to 35	Normal	Normal	Normal	Normal	Normal	0
4	36 to 40	Normal	Normal	Normal	Normal	Normal	0

* Dosage: 6.25 mg. JB-516 per day, orally.

TABLE 11
HEPATITIS*

Subject: A.
Duration: 66 days
Bilirubin: 19
Cephalin flocculation: 4+
Thymol: 28
SGOT: 1100
Alkaline phosphatase (Bodansky): 59

* Dosage: 25 mg. JB-516 per day, orally. Subject showed a strongly positive serum; agglutination of 1:24 against *M. rhesus* cells.

the control (TABLE 7). Of the 3 subjects whose glucose-tolerance tests were repeated after 2 months (TABLE 8), all showed a significant flattening compared with the control group.

Hydroxy indole acetic acid (H.I.A.A.) inhibition. A patient with malignant carcinoid (TABLE 9), whose urine was strongly positive for hydroxy indole acetic acid, was placed on 400 mg. iproniazid per day for 3 days. No decrease in intensity of the urine serotonin derivative was obtained. The iproniazid was followed with 100 mg. per day of JB-516, and a definite but partial inhibition of the metabolite was observed. There is no conclusive evidence that this was due solely to the more potent JB-516; it may have been a cumulative effect of both drugs.

Liver profiles. The sera of 37 subject, all receiving 6.25 mg. of JB-516 per day for 8 to 40 weeks, were examined for possible liver damage (TABLE 10) by conventional clinical chemical methods. Four showed a rise in SGOT values and 1 a rise in the cephalin flocculation. One subject, a diabetic, developed irreversible jaundice (TABLE 11).

Conclusions

A highly potent amine oxidase inhibitor* was studied in 41 humans for its effects on blood sugars, liver function, renal tolerance, urinary bladder, semen urinary steroids, thyroid function, serum electrophoresis, and hemogram.

Two patients developed jaundice, with marked changes in the blood sera.

The drug produced a hypoglycemic glucose-tolerance curve, after 2 months in all 3 patients who had normal patterns after 1 month.

All other measured functions or systems were unchanged, as were the measured functions or systems of a very large majority of patients with respect to such aspects as hemograms and renal functions. Some elevations of serum protein–bound iodine and urinary steroids have yet to be explained.

Our impression of this drug's clinical uses from a therapeutic standpoint as measured by the antianginal response, is that it is about eight times as effective as iproniazid.

References

1. ZELLER, E. A. The Enzymes, J. B. Sumner & K. Myrbäck, Eds. **II:** 536. Academic Press. New York, N. Y.
2. HARE, M. L. C. 1928. Tyramine oxidase. Biochem. J. **22:** 968.
3. PUGH, C. E. M. & J. H. QUASTEL. 1937. Oxidation of aliphatic amines by brain and other tissues. Biochem J. **31:** 2306.
4. DAVISON, A. N. 1958. Physiological role of monoamine oxidase. Physiol Revs. **38** 729.
5. BIEL, J. Personal communication.

Discussion of the Paper

LOUIS GILLESPIE, JR. (*Section of Experimental Therapeutics, National Heart Institute, Public Health Service, Bethesda, Md.*): In our clinical studies in which we used phenylisopropylhydrazine (JB-516, Catron) in daily doses ranging from 6.25 to 50.0 mg., we also followed certain basic laboratory parameters for toxicity. In agreement with Greenberg's report, we have seen n

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* Supplied by H. L. Daiell of Lakeside Laboratories, Inc.

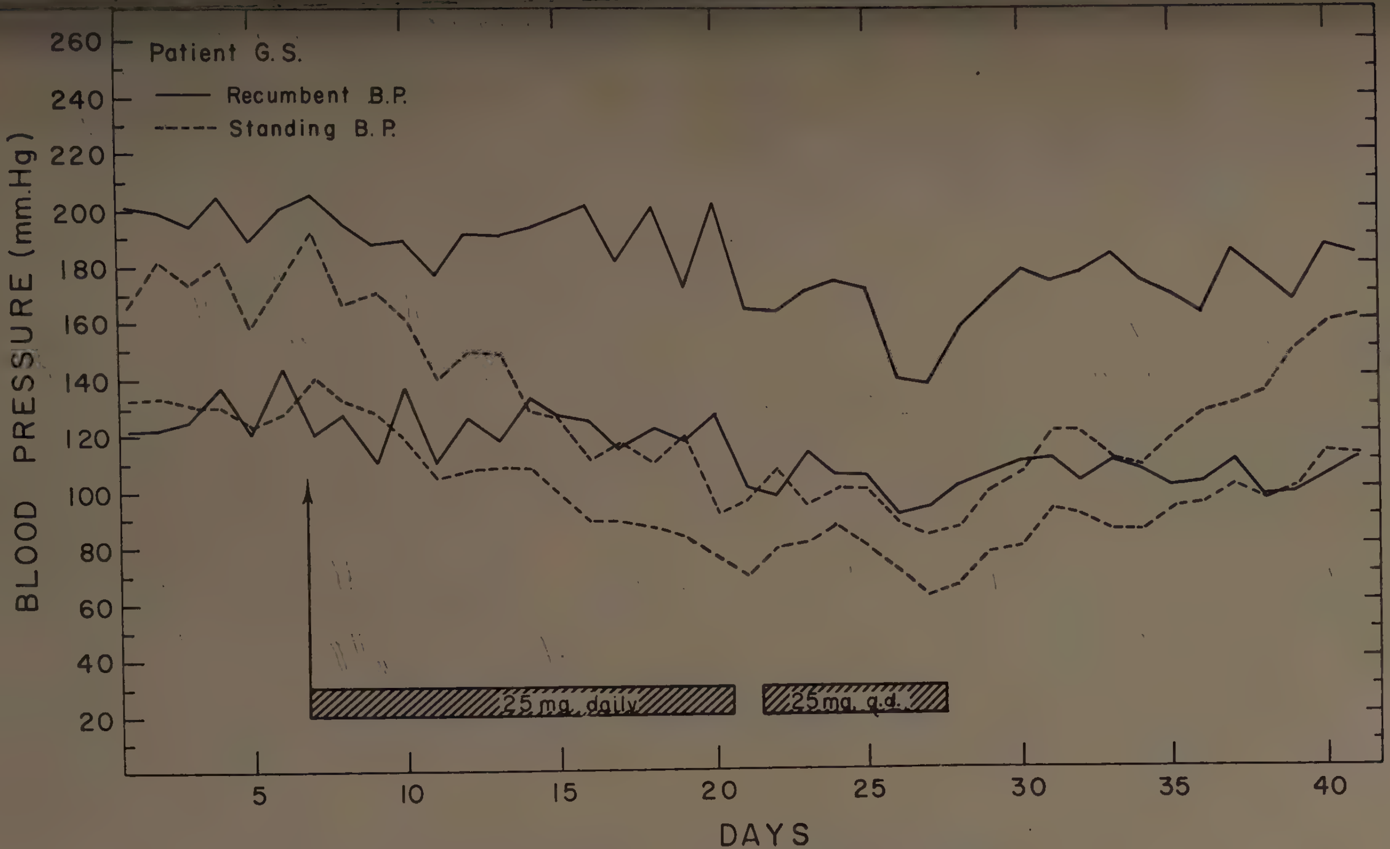

FIGURE 1. Short-term effects of JB-516 on orthostatic blood pressure.

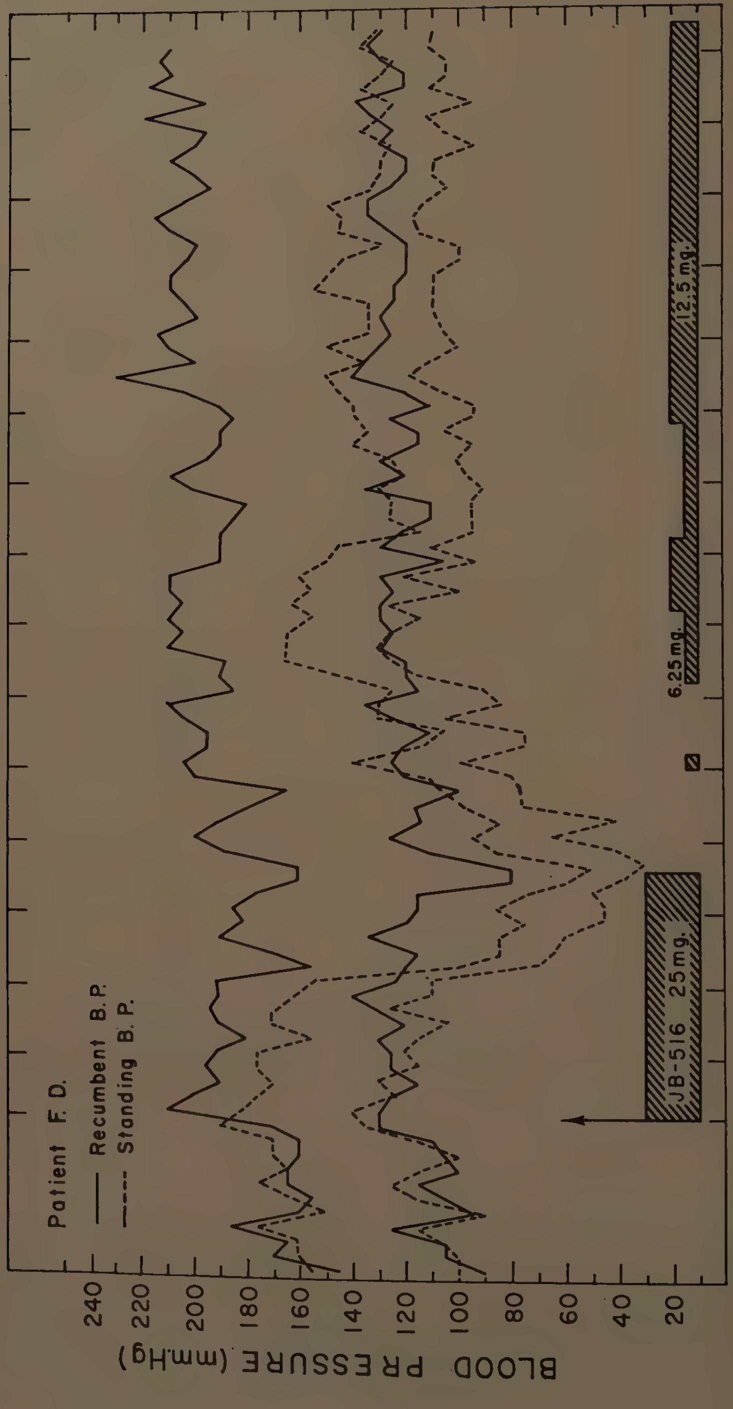

FIGURE 2. Outpatient study with JB-516 alone.

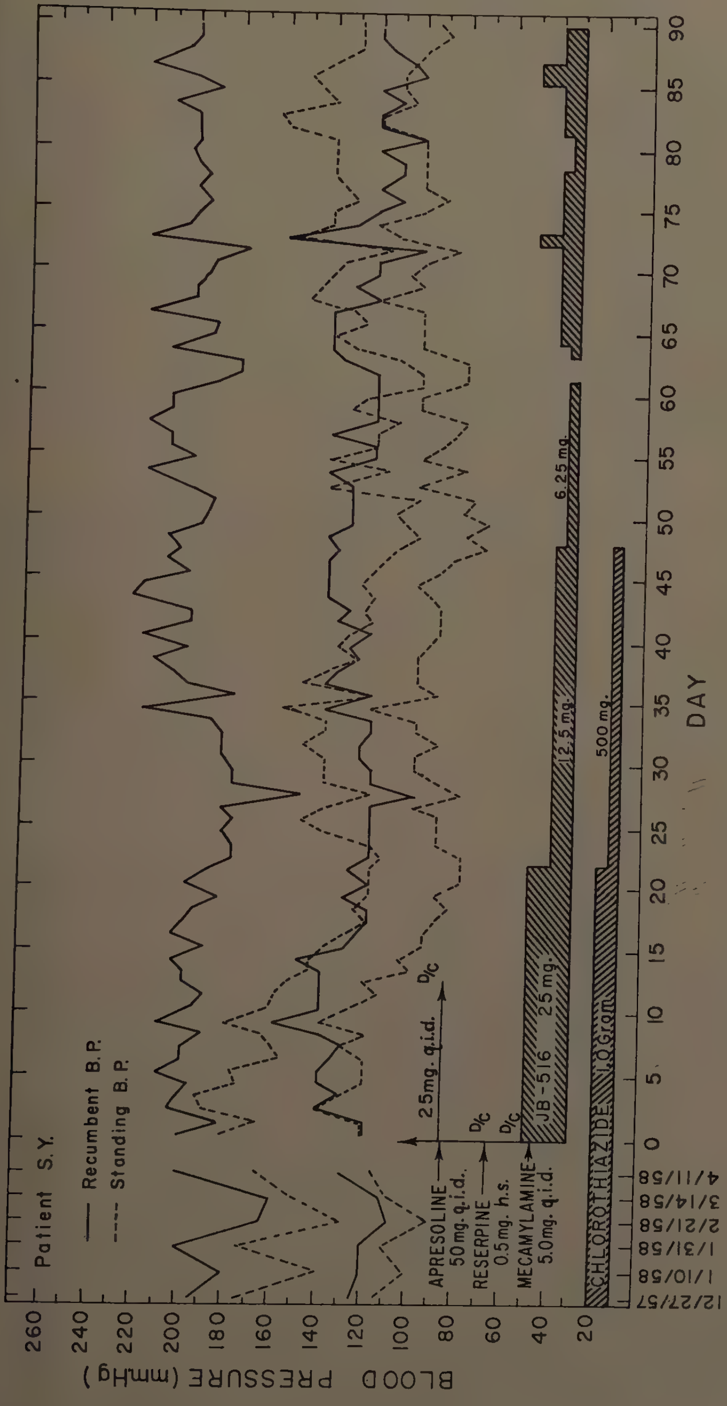

FIGURE 3. Outpatient study with JB-516 in combination with chlorothiazide.

significant abnormalities and, specifically, no indication of alterations in liver function. I take this opportunity to present 2 new facets related to the actions of JB-516 in patients.

An already sizable number of clinical uses for amine oxidase inhibitors is reported in this monograph. I have studied the application of JB-516 in the treatment of moderate to severe primary hypertension. At first I performed short-term studies on 9 hospitalized patients, administering JB-516 in daily doses of 12.5 to 50.0 mg. for periods of 14 to 21 days. Eight of the 9 patients responded with a sustained orthostatic lowering of blood pressure, a typical example of which is illustrated in FIGURE 1. One of the 9 patients received JB-516 in daily doses of 25 to 50 mg. for a 21-day period and failed to show a hypotensive response. The time of onset of the orthostatic lowering of the blood pressure ranged from 3 to 14 days. Following cessation of JB-516 administration, the standing blood pressure begins to rise almost immediately, although up to 15 days may elapse before the standing pressure has returned fully to control levels. There was little effect on the recumbent blood pressure, with the exception of moderate but significant declines in those of patients in the hospitalized group.

TABLE 1
VISUAL SIDE EFFECTS OF JB-516

Patient	Dose (mg./day)	Duration of therapy* (weeks)	Duration of side effect† (weeks)
P.A.	25–50	14	5
B.T.	25	14	4½
E.M.‡	25	19	3
W.E.	25	3	3
E.H.‡	18.75	15	4
S.Y.‡	25	11	5

* Prior to onset of side effect.
† After stopping JB-516.
‡ Restarted on JB-516, 12.5 mg. per day.

Later, I extended my clinical trials to the management of clinic patients wit hypertension. A group of 12 outpatients, 7 of whom had sufficiently sever hypertension to have required ganglionic blocking agents for adequate con trol, has been observed for 2 to 8 months while receiving single daily doses o JB-516 ranging from 6.25 to 25.0 mg. Eleven of the 12 have responded wit sustained lowering of the standing blood pressure. FIGURE 2 shows the re sponse of 1 of our outpatients who had required ganglionic blocking agents but was well controlled on 12.5 mg. daily. FIGURE 3 shows the response o another outpatient who received JB-516 at first in combination with chloro thiazide and later alone, with resultant adequate control of the blood pressure It has been my impression that, when chlorothiazide is administered in com bination with JB-516, potentiation similar to that seen with other antihy pertensive agents is observed.

In addition to the rather striking effects on blood pressure, I have bee impressed with the lack of parasympatholytic effects such as severe constipa tion, difficulty with micturition, dryness of the mouth, blurred vision, and sexual impotence. Two of our male patients, previously impotent while on ganglionic blockade, have regained sexual competence while taking JB-516. My optimism concerning this compound has been enhanced further by the smooth and sustained lowering of the blood pressure observed even when it is administered only once daily.

I have observed an interesting and reversible "side effect" recently in 6 of the 12 outpatients. This consists of the rather sudden onset of loss of red-green color discrimination in association with slight blurring of vision (TABLE 1). It is noteworthy that all of these patients were receiving maximum daily doses (18.75 to 50 mg.). Repeated ophthalmological examinations in all 6 patients failed to demonstrate any objective evidence of decreased visual acuity, alteration of visual fields, or unusual fundiscopic abnormalities. At present, 3 of these patients have been restarted on JB-516 at the smaller dosage of 12.5 mg. daily and are again approaching adequate blood pressure control without recurrence of the color-vision defect.

Finally I, and also others, are continuing the clinical evaluation of JB-516 as an antihypertensive agent. We feel that a drug of this type may largely replace ganglionic blocking agents in the chronic therapy of severe primary hypertension. The fascinating question that arises, of course, is whether a whether a cause-and-effect relationship exists between amine oxidase inhibition and the orthostatic lowering of blood pressure. We are actively seeking an answer to this question at present.

SEROTONIN AND THE DYNAMICS OF THE HEART*

C. W. Crumpton, C. A. Castillo, G. G. Rowe,† G. M. Maxwell
Cardiovascular Research Laboratory and the Department of Medicine,
University of Wisconsin Medical School, Madison, Wis.

Page created the term amphibaric[1] to describe the effects of serotonin upon cardiovascular hemodynamics, the implication being that their trend and magnitude vary with the species, the site of injection, and the dose. In line with this, the present report describes the effects of serotonin upon systemic, pulmonary, and coronary hemodynamics and metabolism in the intact, lightly anesthetized dog. The experiments, directed by George Maxwell, constitute a preliminary report on a larger scale serotonin study still in progress.[2]

Methods

Eighteen mongrel dogs, anesthetized with morphine sulfate (3 mg./kg. intramuscularly) followed in 1 hour by pentobarbital (12 mg./kg. intravenously), exhibited stable arterial blood pressure and respiratory rate, together with a sinus arrhythmia. Catheters were placed in the pulmonary artery, coronary sinus, and the right atrium, as previously described in reports from this laboratory. Control cardiac output (Fick) and coronary blood flow (saturation method, nitrous oxide application of Fick) were determined. Following an interval of 20 min., serotonin creatinine sulfate was administered by infusion via a catheter in the right atrium (20 μg./kg./min.). Five minutes after starting the serotonin infusion, cardiac output and coronary flow measurements were repeated, during which period the infusion was continued (TABLE 1).

Results

Heart rate increased from 94 to 115/min., mean arterial blood pressure decreased from 104 to 85 mm. Hg, and mean pulmonary artery pressure rose by +30 per cent. Since cardiac output did not show a statistically significant change, left ventricular work and total peripheral resistance decreased due to the reduction in mean arterial blood pressure. Total pulmonary resistance and right ventricular work increased as a function of elevation of pulmonary artery pressure. An increase in ventilation was associated with a significant reduction in carbon dioxide content of arterial, mixed venous, and coronary sinus blood. Coronary flow increased from 71 to 85 cc./100 gm./min. Coronary flow/beat ratio was unchanged. Coronary vascular resistance decreased significantly. Coronary sinus venous oxygen content increased from 3.1 to 5.9 vols. per cent. Thus, the decrease in left ventricular oxygen extraction associated with the increase in coronary flow resulted in left ventricular oxygen consumption remaining unchanged. Myocardial carbon dioxide excretion was maintained in a similar manner. The index of efficiency (left ventricular work/left ventricular oxygen consumption) decreased. Coronary blood flow/left ventricular work ratio increased by 65 per cent.

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* The work reported in this paper was supported in part by grants from the National Hear Institute, Public Health Service, Bethesda, Md., the Wisconsin Heart Association, Milwaukee Wis., and the Wisconsin Alumni Research Foundation, Madison, Wis.

† John and Mary R. Markle Scholar in Medicine of the University of Wisconsin.

Discussion

The literature concerning the actions of serotonin confirms the thesis of variation according to species, site of injection, and concentration administered per unit of time. Under the experimental conditions described in this report, serotonin acts as a peripheral and coronary vasodilator and has a vasoconstrictor effect upon the pulmonary vascular system.

Following serotonin infusion (20 μg./kg./min.), cardiac output did not change while coronary blood flow increased +20 per cent. Since the heart rate increased from 94 to 115/min., the question arises as to the influence of the tachycardia on coronary blood flow. Previous studies[3] in this laboratory have shown that with increases in heart rate from a mean control of 92/min. to 189/min., cardiac output was unchanged while coronary flow rose from 81 to 148 cc./100 gm./min. While the trend of the effect upon flow was similar in the serotonin and the induced tachycardia studies, there was a well-defined difference in the oxygen metabolic pattern, the left ventricular oxygen consumption remaining constant following serotonin infusion. This was accomplished by the increase in coronary flow being compensated by a reduction in the amount of oxygen extracted by the left ventricle per unit of time. In the induced-tachycardia study there was a highly significant increase in left ventricular oxygen consumption due to (1) the increase in coronary blood flow and (2) the fact that at a faster heart rate the left ventricle continued to extract the same amount of oxygen as at the slower basal control rate. These observations suggest that the tachycardia associated with the infusion of serotonin was not a primary contributing factor to the observed systemic and coronary hemodynamic and metabolic changes.

TABLE 1

EFFECTS OF SEROTONIN (20 μG./KG./MIN.) IN DOGS

	Control	Experimental	Per cent change
Heart rate (min.)	94	115	+21*
Cardiac output (l./min.)	2.6	2.6	0
Mean arterial blood pressure (mm. Hg)	104	85	−18*
Total peripheral resistance (dynes/sec./cm.$^{-5}$)	3565	2824	−21*
Mean pulmonary arterial pressure (mm. Hg)	10	13	+30*
Total pulmonary resistance (dynes/sec./cm.$^{-5}$)	345	471	+36*
Coronary blood flow (cc./100 gm./min.)	71	85	+20
Coronary vascular resistance (units)	1.5	1.1	−27*
Respiratory volume (l./min.)	3.1	3.4	+10*
Coronary sinus O_2 content (Vols. per cent)	3.9	5.1	+31*
Left ventricle O_2 consumption (cc./100 gm./min.)	9.0	9.4	+4

* Denotes statistically significant change $p < 0.05$.

The respiratory response following serotonin has been clarified by McCubbin *et al.*[4] by showing that small doses of serotonin fail to stimulate respiration unless the drug reaches chemoreceptors. Since much larger doses after section of the sinus nerve caused the reappearance of a respiratory response, the mechanism was assumed to be central. It persisted after cervical section of the spinal cord and section of the vagus-aortic and sinus nerves. Under the experimental conditions described in this report, while respiratory volume increased, oxygen consumption and carbon dioxide production did not change significantly.

Mean arterial blood pressure decreased by 18 per cent when compared with the mean average control value. Since the flow did not change, the decrease in calculated total peripheral resistance was due to the reduction in pressure. The rise in heart rate caused a 14 per cent decrease in stroke volume, but this reaction was of no statistical significance.

Mean pulmonary artery pressure increased from a control of 10 mm. Hg to 13 mm. Hg following the serotonin infusion. This increase, although quantitatively small, was highly significant, since it occurred regularly in all 18 animals studied. Since cardiac output did not change, the increase in right ventricular work and total pulmonary resistance was a function of the rise in mean pulmonary artery pressure.

The coronary vascular resistance (pressure/flow ratio) decreased due to changes in both mean arterial blood pressure (↓) and left ventricular blood flow (↑). The coronary blood flow/beat remained unchanged. Gregg has defined "benign" and "malignant" coronary dilatation.[5] Serotonin increased coronary blood flow and reduced oxygen extraction by the left ventricle, thus maintaining left ventricular oxygen consumption unaltered.

To determine the effects of serotonin on peripheral blood pressure, Haddy *et al.*, infused serotonin into the brachial arteries of 35 dogs with forelimb vessels normal, constricted, and dilated.[6] They demonstrated that serotonin can be either a constrictor or a dilator, and that it antagonizes extremes of neurogenically induced vascular tone. Their studies confirmed the fact that serotonin may provide the body with a chemical buffering system. From these studies Haddy and his co-workers concluded that the irregular effect of serotonin upon arterial pressure may in part be related to the varying degree of neurogenic tone present at the time of administration.[6]

Summary

The hemodynamic action of serotonin may vary according to the species, the site of injection, the dose concentration, and the degree of neurogenic tone existing at the time of administration.

Under the experimental conditions described, serotonin acts as a peripheral and coronary vasodilator and has a vasoconstrictor effect upon the pulmonary vascular system.

The decrease in coronary vascular resistance was associated with a reduction in mean arterial blood pressure and an increase in coronary blood flow, with left ventricular oxygen consumption remaining unchanged. This is the pattern of a "benign" coronary vasodilator.

References

1. Page, I. H. 1954. Serotonin (5-hydroxytryptamine). Physiol. Revs. **34:** 563–588.
2. Maxwell, G. M., C. A. Castillo, D. H. White, Jr. & C. W. Crumpton. 1957. Th

effects of serotonin (5-hydroxytryptamine) upon systemic, pulmonary, and coronary hemodynamics and metabolism. J. Lab. Clin. Med. **50:** 930–931 (Abstract).

3. Maxwell, G. M., C. A. Castillo, D. H. White, Jr., C. W. Crumpton & G. G. Rowe. 1958. Induced tachycardia: its effect upon the coronary hemodynamics, myocardial metabolism and cardiac efficiency of the intact dog. J. Clin. Invest. **37:** 1413–1418.
4. McCubbin, J. W., J. H. Green, G. C. Salmoiraghi & I. H. Page. 1956. The chemoceptor stimulant action of serotonin in dogs. J. Pharmacol. Exptl. Therap. **116:** 191–197.
5. Gregg, D. E. & D. C. Sabiston, Jr. 1956. Current research and problems of the coronary circulation. Circulation. **13:** 916–927.
6. Haddy, F. J., D. A. Emanuel, P. Gordon, J. Scott, M. Fleishman & M. Lipton. 1958. Serotonin—an antagonist to extremes of neurogenic vasomotor tone. Abstr.. Proc. Central Soc. Clin. Research. **31:** 35.

Discussion of the Paper

I. Leusen (*Laboratory of Physiopathology, University of Ghent, Ghent, Belgium*): I congratulate Crumpton and his colleagues for their work on the influence of serotonin (5-HT) on the dynamics of the dog heart, and I shall endeavor to make some points relating to their results.

The influence of serotonin on the pulmonary circulation is very pronounced, small doses producing a significant increase in pulmonary arterial pressure. In this respect the influence of serotonin is more pronounced than that of any other of the known vasoactive principles, including epinephrine and norepinephrine.

The effect of 5-HT on general arterial blood pressure is much less uniform. Although in isolated vascular preparations this substance produces constriction, the over-all effect on pressure is more variable. Species differences in sensitivity and type of reaction, as well as wide variations of response in the same species have been described. The reaction to serotonin depends on factors such as the state of the animals, whether they are anesthetized, and the kind of anesthesia employed. The degree of existing central nervous vasomotor tone is also important in determining the kind of blood pressure reaction obtained. Page uses the term amphibaric to describe the variability of the blood pressure reaction to 5-HT.[1]

In studies using isolated perfused hearts a positive inotropic and chronotropic effect of 5-HT was regularly produced, showing a direct effect of serotonin on the hearts of cats, rabbits, and dogs. Through use of the isolated right auricle preparation of rabbit and cat hearts, this chronotropic and inotropic effect has been generally confirmed.

In studies recently performed in our laboratory a positive inotropic effect of 5-HT on the isolated papillary muscle from the right side of the cat heart was also regularly observed.

We may conclude that the effects of serotonin on the cardiovascular system and, more precisely, on the general arterial blood pressure are decidedly variable. Many factors probably play a role in this cardiovascular effect.

Difficulties of a different kind arise in animal studies on the cardiovascular influence of iproniazid. In patients with angina pectoris and some others, the clinical effects of iproniazid on arterial blood pressure are based upon gradual and progressive medication with the drug; consequently, it is difficult to extrapolate observations made with high doses in acute animal experiments to the more prolonged clinical effect of the substance. On the other hand, chronic studies concerning the cardiovascular effects of iproniazid are difficult to achieve. Since experimental work on the cardiovascular effects of iproniazid (studies of effects on isolated perfused hearts and on intravascular pressures)[2, 3] is rather scarce, I shall summarize some results obtained by the injection of iproniazid in anesthetized dogs in acute experiments.

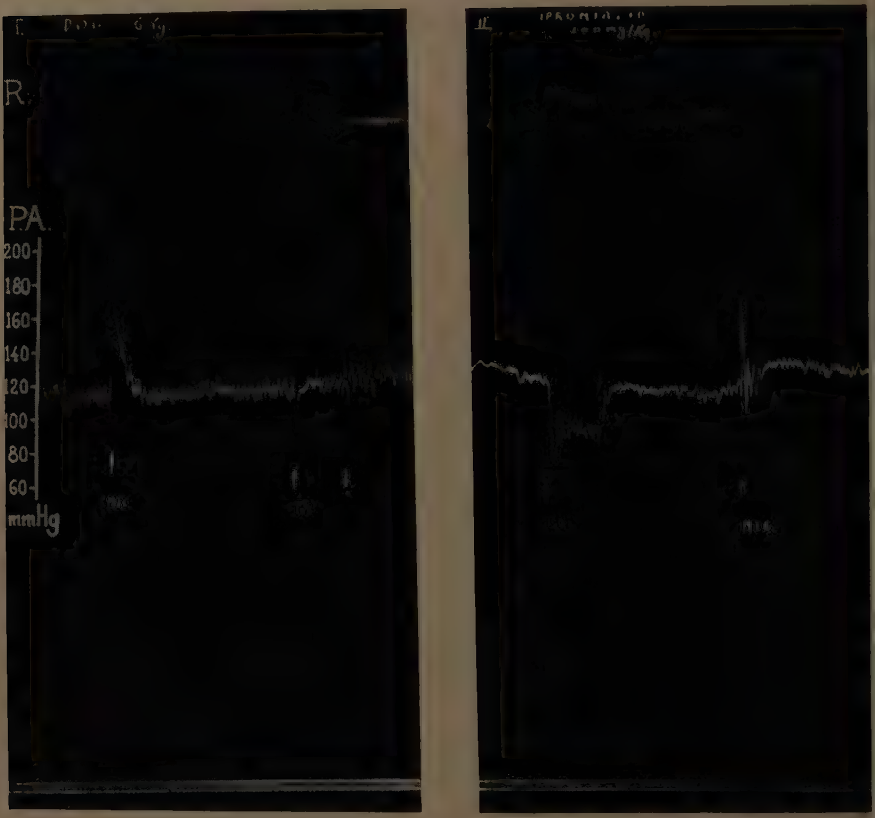

FIGURE 1. Changes in blood pressure in a 6-kg. dog induced by the slow intravenous injection of iproniazid (100 mg./kg.). *Left,* normal; *right,* after injection.

Methods

The experiments were performed in dogs under morphine (0.5 mg./kg.)-chloralose (0.08 mg./kg.) anesthesia. In some animals the vagi were cut. Specific procedures employed will be described in the appropriate places. The substance used was the phosphate salt of iproniazid (Marsilid).

Results

In intact anesthetized dogs the blood pressure did not change markedly after the slow intravenous injection of iproniazid phosphate in doses as high as 100 mg./kg. Except for a small increase, cardiac output also was not usually changed substantially. On the other hand, the heart rate rose markedly. In spite of the relatively small changes in blood pressure, cardiovascular adaptation reactions were depressed and were manifested on tilting the animal. In contrast with control conditions (FIGURE 1), iproniazid phosphate caused a more pronounced initial decrease of the blood pressure that did not return to its original level as long as the vertical position was maintained.[4]

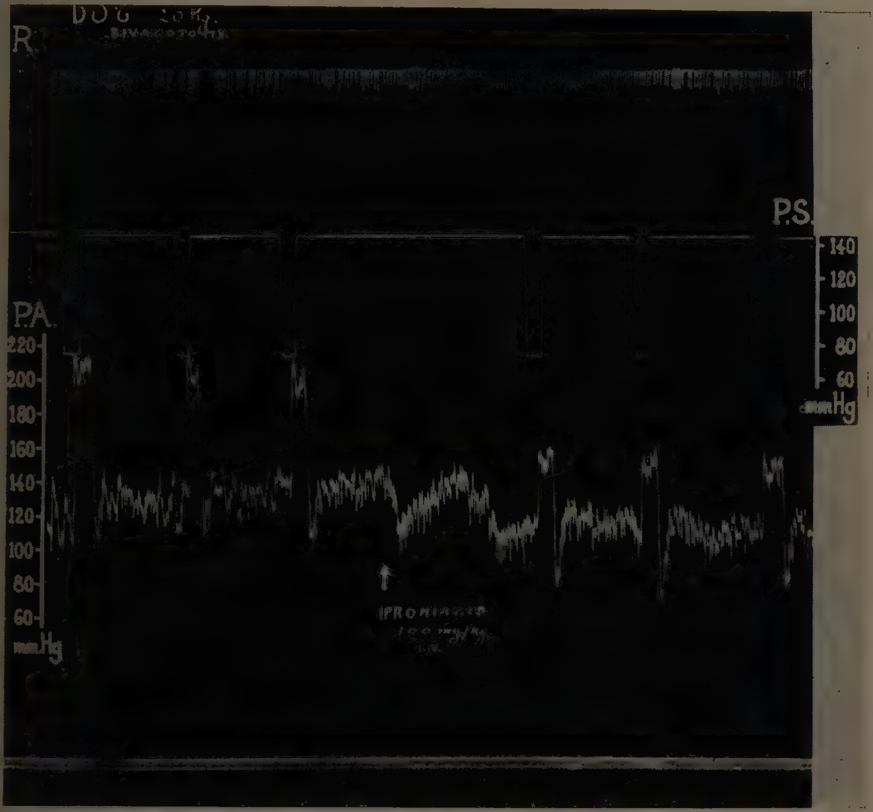

FIGURE 2. Effect of the intravenous injection of iproniazid (100 mg./kg.) on a 20-kg. bivagotomized dog.

In bivagotomized dogs the blood pressure regularly showed a long-lasting fall after slow intravenous injection of the substance in doses of 50 to 100 mg./kg. Simultaneously, the carotid sinus reflexes evoked by clamping both carotid arteries were clearly depressed. This depression of the carotid reflexes was also clear in experiments with isolated but normally innervated carotid sinuses when the pressure in the isolated sinuses was changed (FIGURE 2).

A rather significant depression of the cardiovascular effects of epinephrine and norepinephrine was observed; this confirmed the observations made in the cat by Schmitt and Gonnard.[4] The hypertension induced by the injection of nicotine (0.5 mg.), known to be caused by stimulation of ganglionic synapses, the chemoreceptors, and by the norepinephrine discharged from the adrenal medulla, also was markedly depressed after iproniazid (FIGURE 3). The hypertension by high doses of acetylcholine (2 mg.) in the atropinized animal that is produced by the same mechanism as the nicotine hypertension was also markedly depressed after iproniazid (FIGURE 4).

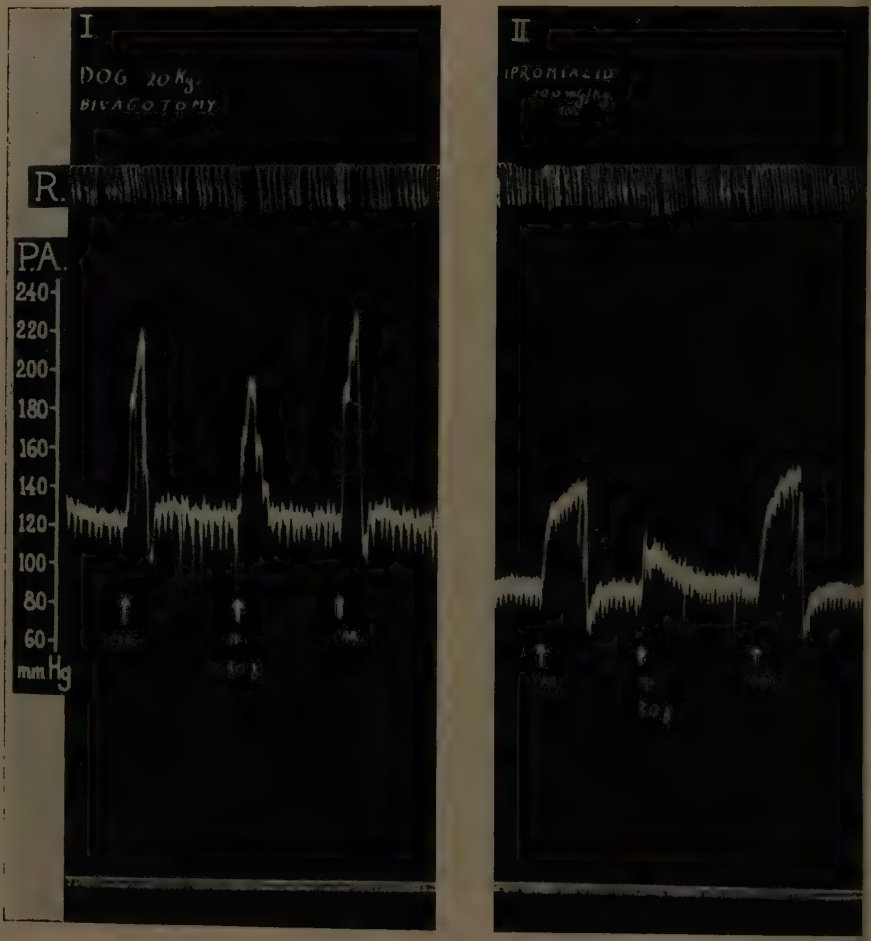

FIGURE 3. Hypertension induced by the injection of nicotine in a 20-kg. bivagotomized dog. *Left*, before iproniazid; *right*, after the intravenous injection of iproniazid (100 mg./kg.).

These experiments under acute conditions indicate possible ways by which the cardiovascular system may be depressed by treatment with iproniazid. Possibly the mechanism may be a decreased reactivity of the periphery to constrictive humoral or nervous stimuli. Griesemer *et al.*[5] have shown that iproniazid diminishes the epinephrine-induced contraction of the smooth muscle of rabbit aorta. A central mechanism can also play a role. These effects however are seen in acute experiments only after high doses of iproniazid (50 to 100 mg./kg.). The question remains whether there is any connection between these pharmacological findings and the clinical effect of the drug.

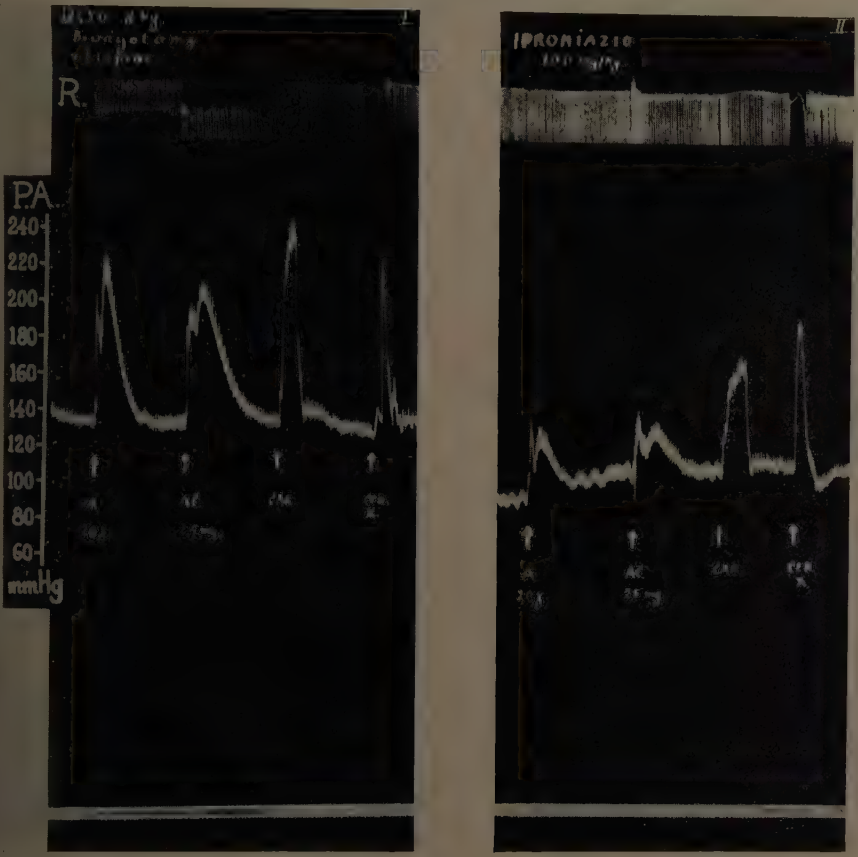

FIGURE 4. Hypertension induced by acetylcholine in an 8-kg. bivagotomized and atropinized (2 mg.) dog. *Left,* before iproniazid; *right,* after injection of iproniazid (100 mg./kg.).

Summary

In acute experiments in morphine-chloralose anesthetized dogs, a depression of the carotid sinus reflexes and of the pressor effects of adrenaline, noradrenaline, nicotine, and acetylcholine (after atropinization) was observed after iproniazid injection (50 to 100 mg./kg). The compensating mechanisms for maintaining blood pressure on tilting were also depressed after iproniazid.

Acknowledgment

The iproniazid used in these studies was provided by F. Hoffmann-La Roche & Co., Ltd., Basle, Switzerland.

References

1. Page, I. H. 1954. Serotonin (5-hydroxytryptamine). Physiol. Revs. **34:** 563–588.
2. Almark, M. G., F. C. Lu, E. Carmichael & A. Lavallee. 1953. Some pharmacological observations on isoniazid and iproniazid. Am. Rev. Tuberc. **68:** 199.
3. Pletscher, A. & B. Pellmont. 1958. Biochemical and pharmacologic actions of Marsilid on the heart. J. Clin. Exptl. Psychopathol. **19**(2), Suppl. **1:** 163.
4. Schmitt, H. & P. Gonnard. 1956. Modifications par un inhibiteur de l'aminoxydase, l'iproniazide, des effets de quelques amines sympathicomimetiques sur la membrane nictitante du chat. Arch. intern. pharmacodynamie. **108:** 74.
5. Griesemer, E. C., C. A. Dragstedt, J. A. Wells & E. A. Zeller. 1955. Adrenergic blockade by iproniazid. Experientia. **11:** 182.

A METHOD FOR MEASUREMENT OF MONOAMINE OXIDASE INHIBITION IN MAN: APPLICATION TO STUDIES ON HYPERTENSION

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Section of Experimental Therapeutics and Laboratory of Clinical Biochemistry, National Heart Institute, Public Health Service, Bethesda, Md.

It is important to ascertain whether drugs which, on the basis of studies on animals, are called monoamine oxidase (MAO) inhibitors, do indeed inhibit this enzyme(s) in man in ordinary clinical dosage. To help solve this problem, a test was devised whereby this enzymatic effect of a drug can be measured in the human.[1] The test is based on the conversion of orally administered serotonin to 5-hydroxyindoleacetic acid (5-HIAA), which is the end product of MAO activity on this amine.

From a clinical standpoint, the effects of MAO inhibitors on the cardiovascular system have been of particular interest to us. Since MAO is involved in the metabolism of several vasoactive monoamines, alterations in cardiovascular functions might be anticipated. It is noteworthy that iproniazid (Marsilid*) has been found to be useful in the treatment of angina pectoris[2] and also hypertension[3, 4] in some patients. Certain other MAO inhibitors also appear to have the property of lowering blood pressure in man.

This presentation is concerned chiefly with the problem of measuring MAO inhibition in man and the relationship of MAO inhibition to alterations in the blood pressure of patients with hypertension.

Procedure

Following oral administration of an arbitrary dose of 20 mg. of serotonin in a capsule, urine is collected for an 8-hour period and assayed for 5-HIAA. Repetition of this procedure on several successive days establishes a control range of conversion to 5-HIAA, usually 18 to 22 mg. per 8 hours. Thus, 80 to 100 per cent of the serotonin is excreted as 5-HIAA normally. This procedure is continued during a period when a presumed MAO inhibitor is administered. Each drug is administered on a regular schedule, stipulating only that a dose be given 2 hours preceding the administration of serotonin each day. Doses of serotonin as high as 50 mg. have been given orally to patients without apparent effect, even in the presence of MAO inhibition.

Effects of Various Drugs on MAO

The results obtained with drugs, which were tested both *in vitro* and in man for MAO-inhibitory action, are shown in TABLE 1. The agents are listed in order of decreasing potency *in vitro*. Harmaline, a *harmala* alkaloid, is an example of a short-acting inhibitor, while 1-phenyl-2-hydrazinopropane (JB-516) and iproniazid are examples of long-acting MAO inhibitors. All three drugs markedly reduced the conversion of serotonin to 5-HIAA in patients. Procaine amide and methoxyphenamine (Orthoxine[*]) are potent inhibitors *in vitro*, but they exhibited only slight activity in man by the serotonin-metabolism test. Although amphetamine and ephedrine are frequently referred to as MAO inhibitors, they have relatively weak activity *in vitro* and are totally inactive in ordinary clinical dosage as measured in our procedure. The remaining drugs are commonly used cardiovascular agents; none had significant activity on MAO. In the course of these studies it was also noted that barbiturates, salicylates, and cardiac glycosides did not affect the amount of 5-HIAA derived from the administered serotonin.

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* Hoffmann-La Roche.

TABLE 1

EFFECT OF VARIOUS DRUGS ON MAO IN MAN AND IN HOMOGENATES OF RAT LIVER

Drugs[*]	Dose (mg.) interval (hours)	Inhibition of serotonin to 5-HIAA in man (percentages)	Conc. producing approx. 50 per cent inhibition *in vitro*[†]
Harmaline	75/8	70 to 80	10^{-6} M
JB-516	25/24	70 to 80	5×10^{-6} M
Iproniazid	100/8	50 to 70	3×10^{-4} M
Procaine amide	500/6	25	10^{-4} M
Orthoxine	100/4	20	10^{-4} M
Amphetamine	10/2	0	10^{-3} M
Ephedrine	100/2	0	$>10^{-3}$ M
Quinidine	200/6	0	$>10^{-3}$ M
Nitroglycerine	0.6/1	0	$>10^{-3}$ M
Peritrate	20/6	0	$>10^{-3}$ M
Chlorothiazide	500/6	0	$>10^{-3}$ M
Mecamylamine	5/6	0	$>10^{-3}$ M
Hydralazine	50/6	0	$>10^{-3}$ M
Reserpine	1.0/12	0	$>10^{-3}$ M

[*] Nitroglycerine given sublingually; other drugs ingested orally.

[†] MAO activity measured by serotonin-disappearance technique.

Site of MAO Inhibition Measured by the Test

Although a diminution of 5-HIAA production from orally administered serotonin might be indicative of an over-all MAO inhibition with a particular drug, it was felt that only the inhibition occurring in the gut and possibly the liver was being measured by the test. Several experiments were done to study this problem. No increase in the level of blood serotonin could be found in several patients after ingestion of up to 50 mg. of the amine, either in control status or during administration of JB-516. The blood levels of total 5-hydroxyindoles rose significantly; however, this rise was presumably due to increased 5-HIAA. In rats given 12 mg./kg. of serotonin by stomach tube, the blood level of the amine rose from 0.6 μg./ml. to 3.0 μg./ml., but the concentration in the liver was unchanged from control values (av., 1.0 μg./gm.). If JB-516 (5 mg./kg.) was administered 18 hours previously, a similar rise in the blood level was observed in 1 hour but, in addition, the concentration in the liver was about twice that of the controls; hence it appears that only small amounts of serotonin given orally gain access to the systemic circulation.

The possibility remained that the MAO inhibitors produce a decrease in urinary 5-HIAA by interfering with absorption of 5-hydroxyindoles from the gut. Accordingly, the fate of serotonin-2-C^{14} was studied in 2 patients at a time when 5-HIAA formation was inhibited by about 75 per cent with JB-516. Five μc. was given orally in one case and intravenously in the other. Of the counts administered, 70 to 80 per cent were excreted in the urine within 24 hours. Thus, the possibility of poor absorption to account for marked degrees of inhibition was eliminated. Of the radioactivity appearing in the urine during the first 8 hours, 26 per cent was present in 5-HIAA in the oral study, and 63 per cent after injection intravenously. The MAO blockade induced by JB-516 was more effective, therefore, in altering the metabolism of serotonin given orally. This finding is fortunate in one sense since it may lend greater sensitivity to the oral test. However, because of this shortcoming in the oral serotonin test in measuring over-all MAO inhibition, we have explored other approaches. As Oates mentions elsewhere in this monograph, urinary tryptamine is a very sensitive index of over-all amine oxidase activity. This test offers considerable promise as a method of evaluating amine oxidase inhibition in man.

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[*] Upjohn.

It has been shown by Weissbach *et al.*[5] that O-glucuronidation was an alternate and effective pathway for disposal of serotonin administered to rodents when MAO was blocked with iproniazid. In "blocked" patients we have been unable to account for that portion of the serotonin that is not converted to urinary 5-HIAA. The missing fraction could not be recovered in the urine as serotonin itself, the O-glucuronide, or any acid-hydrolyzable conjugate. Radioactive metabolites in the urine of the patients who received serotonin-2-C^{14} are currently being identified.

Vascular and Enzymatic Actions of MAO Inhibitors in Patients with Hypertension

Thus far detailed studies have been done only with iproniazid, JB-516, and harmaline.

Iproniazid. It is now generally known that orthostatic hypotension was a common effect of this drug in patients who received daily doses of 150 mg. A few favorable reports[3, 4] have appeared on the use of this agent as an antihypertensive. In a group of 3 hospitalized hypertensives treated with 150 to 600 mg./day, we were unable to produce consistent lowering of the standing blood pressure during short-term therapy. Where the hypotensive response was satisfactory, effective therapy often could not be maintained because of toxic effects, chiefly neuromuscular hyperexcitability. Recently Orvis and Tamagna (personal communication) have found that the combination of chlorothiazide (1.0 gm./day) with iproniazid (75 mg.) constituted an effective antihypertensive regimen in a small group of clinic patients. It remains to be determined whether addition of 50 mg. of iproniazid per day—the currently recommended dose for ambulatory patients—will offer any advantage over chlorothiazide alone.

We were curious to learn whether the presence or absence of a hypotensive response to iproniazid might be due to individual variations in MAO inhibition. FIGURE 1 illustrates the enzymatic effect in a patient in whom no hypotensive effect was produced. The inhibition of serotonin conversion to 5-HIAA shown

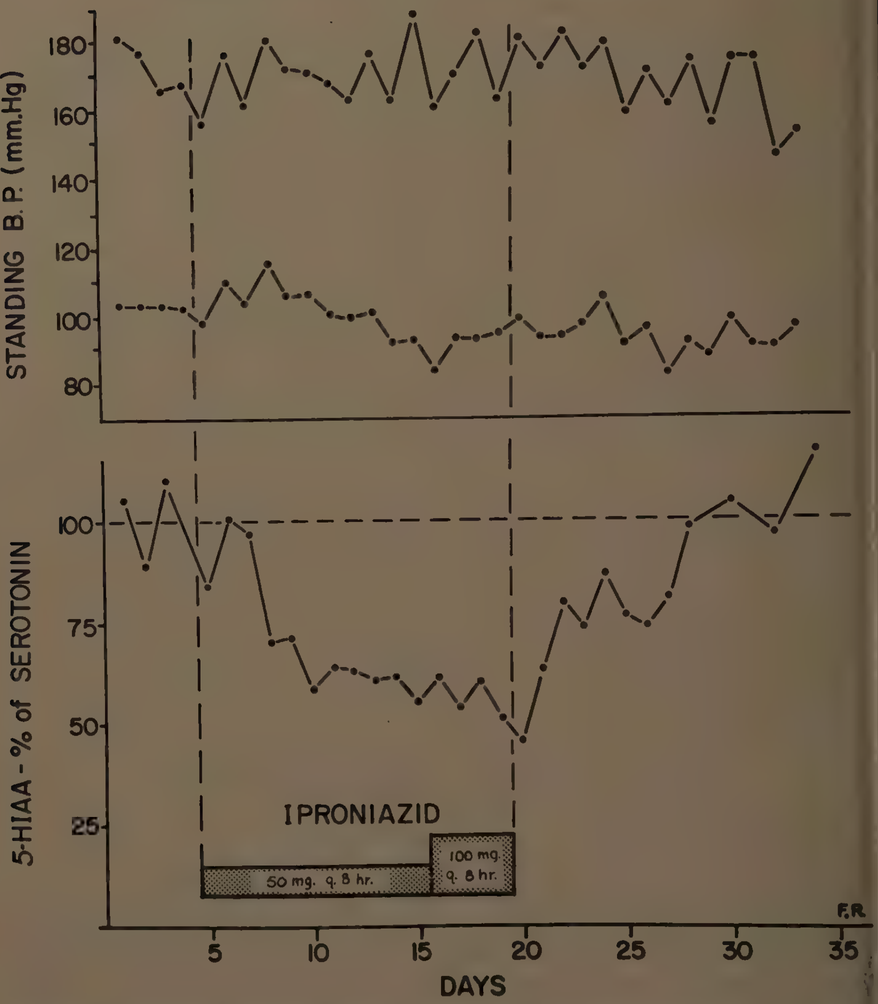

FIGURE 1. Effects of iproniazid on blood pressure and conversion of serotonin to 5-HIAA.

TABLE 2
ENZYMATIC AND BLOOD PRESSURE EFFECTS OF IPRONIAZID

Patient	Av. sitting blood pressure (mm. Hg)		Inhibition of serotonin to 5-HIAA† (percentages)
	Placebo	Iproniazid*	
C.G.	205/128	185/109	40
H.J.	182/137	115/81	44
M.H.	168/104	178/104	43
M.W.	180/130	132/92	55
L.M.	187/111	141/95	33

* Maintenance therapy with 150 mg./day except for patient H.J. who received 75 mg./day

† Average of at least 2 tests.

here was close to the maximum we have attained with the drug. On the other hand, other patients have had a lowering of the blood pressure with a comparable or even lesser degree of inhibition. Some examples of this are shown in TABLE 2.

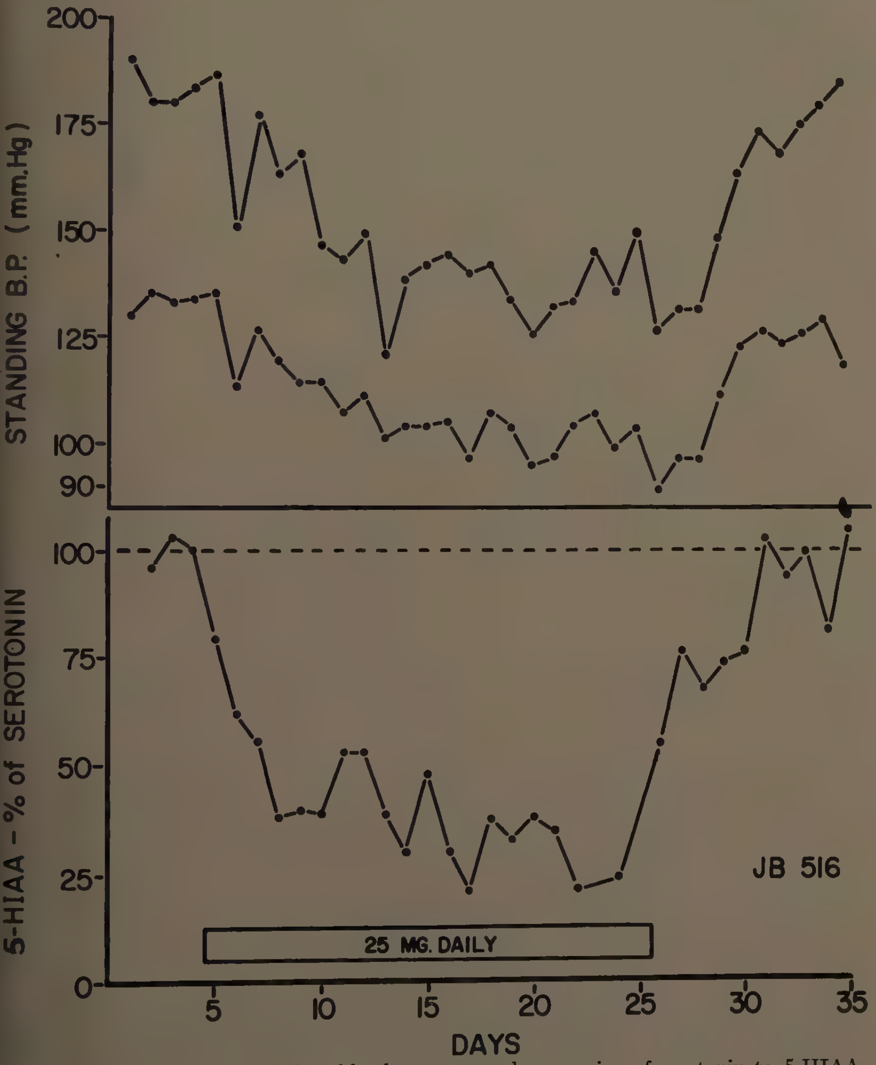

FIGURE 2. Effects of JB-516 on blood pressure and conversion of serotonin to 5-HIAA.

1-Phenyl-2-hydrazinopropane (*JB-516*). A supply of this compound was obtained early this year for enzymatic studies in man. Our plan to perform such studies in hypertensives was viewed with skepticism since the drug was known to be a potent pressor agent in the dog. Its effects on MAO and blood pressure in the first patient studied are shown in FIGURE 2. There was a striking decrease in blood pressure in the upright position that paralleled the chemical effect. Studies were then gradually extended to other patients. The conversion of serotonin to 5-HIAA, as well as the blood pressure in the supine and upright position, were measured during short-term therapy with the drug in 9 hospitalized patients. In each case, enzyme inhibition comparable to that shown in FIGURE 2 was observed. Generally there was little change in the supine blood pressure; however, as shown in TABLE 3, with one exception (patient R.A.M.), there was a uniform and significant orthostatic lowering of the blood pressure. The drug has now been administered to a larger group of patients for periods up to 8 months; the favorable therapeutic results obtained have already been presented elsewhere.[6] The only significant toxic effect has been the development of temporary red-green color blindness at high dose levels (25 mg./day), as discussed by Gillespie elsewhere in this monograph. Most patients do not require a dose of this magnitude. It is our current recommendation that, after lowering of the blood pressure is achieved, the patient be maintained on a single daily dosage of 12.5 mg./day or less, alone or with chlorothiazide. Clinically, the action of the drug on blood pressure is similar to that of the ganglionic blocking agents, but it has the apparent advantages of continuous control with a once-a-day dose and of not producing the side effects of sexual impotency, mydriasis, and severe constipation.

TABLE 3

AVERAGE BLOOD PRESSURE (MM. HG) BEFORE AND DURING THERAPY WITH JB-516

Patient	Daily dose of drug (mg.)	Duration of therapy (days)	Supine B.P.		Standing B.P.	
			Before	During	Before	During
G.S.	25	21	200/130	170/105	173/130	100/75
W.P.	25	21	195/135	200/140	180/130	130/100
N.H.	25	23	210/125	175/115	205/135	110/90
P.A.	25–50	12	210/135	200/110	190/125	120/85
R.M.	25–50	12	140/105	150/110	140/105	105/80
R.A.M.	25–50	25	175/115	180/120	175/115	180/120
S.Q.	12.5–25	21	210/125	215/125	220/130	140/95
V.S.	25	14	200/110	180/95	200/120	100/65
Z.G.	25	14	195/125	200/125	180/125	120/80

Harmaline. It is of interest that harmaline, harmine, and other *harmala* alkaloids were used therapeutically more than 20 years ago in various disorders for which iproniazid and other MAO inhibitors are now being suggested. The excellent review by Gunn[7] in 1935 discusses the hypotensive and central excitatory properties of these alkaloids, as well as apparent usefulness in the therapy of angina pectoris and Parkinsonism. This was before MAO was even known. Recently, it was found that certain *harmala* alkaloids are among the most potent reversible inhibitors of MAO.[8]

Since almost all the MAO inhibitors currently available are hydrazines, it has been impossible thus far to separate clearly effects that are due to MAO inhibition from those that depend simply on the hydrazine group. An agent such as harmaline, which differs radically in chemical structure from the hydrazine compounds, offered promise in this respect. Accordingly, harmaline was administered in a dosage of 75 mg. every 8 hours to 5 hypertensives for 7 to 14 days; a typical response is shown in FIGURE 3. In each case there was a marked inhibition of serotonin metabolism to 5-HIAA in the absence of any significant effect on the blood pressure. In contrast to the slow onset and offset of MAO inhibition as seen with iproniazid and JB-516, maximal inhibition with harmaline was apparent after only one dose of the drug, and no evidence of enzyme inhibition remained on the day after therapy was discontinued.

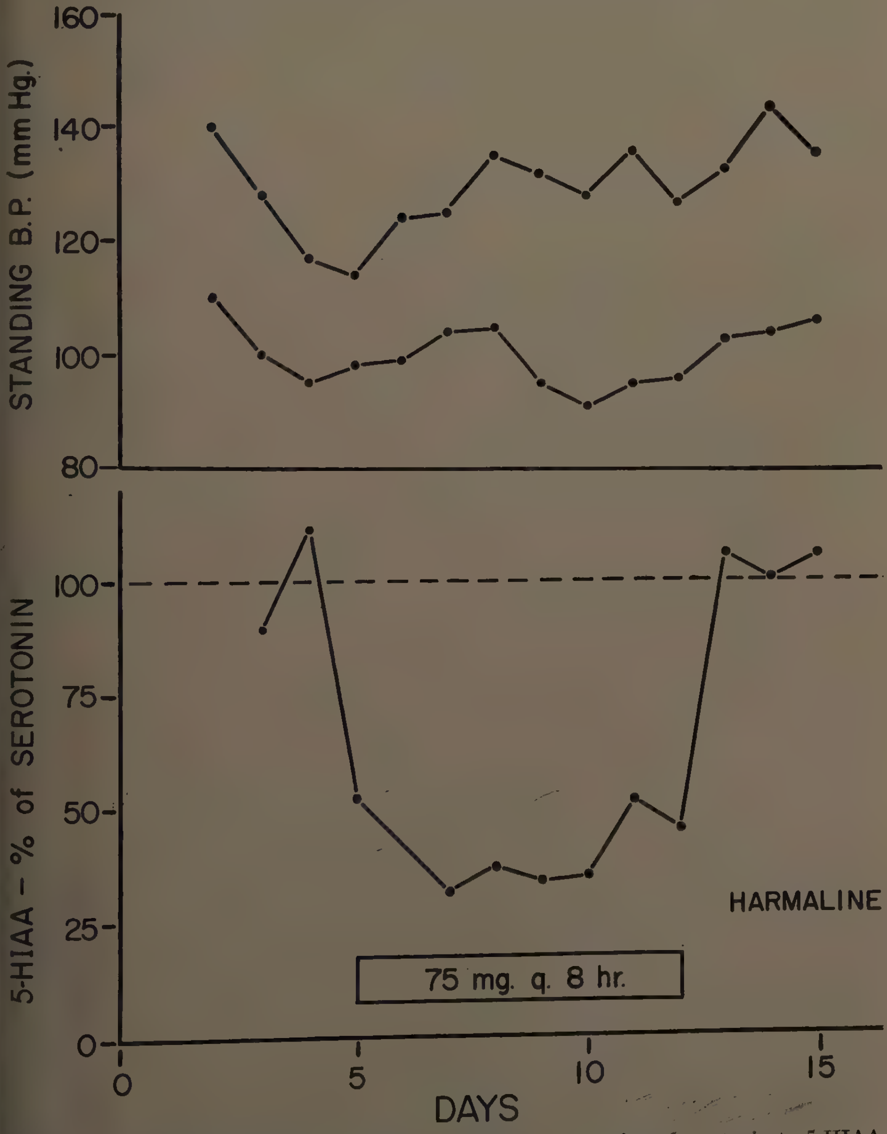

FIGURE 3. Effects of harmaline on blood pressure and conversion of serotonin to 5-HIAA.

At first glance, the results obtained with harmaline would seem to prov that a decrease in blood pressure is not a necessary concomitant of MA inhibition. However, this conclusion is not yet warranted. Available pharmacological evidence, as well as preliminary drug metabolism studies in thi laboratory, suggest that the harmala alkaloids are absorbed incompletely from the gut (Hess, unpublished observations). Thus, it is conceivable that inhibition could be quite marked in the gut, but MAO inhibition elsewhere be inconsequential. Also, since harmaline is a short-acting reversible inhibitor, MA inhibition may have been of insufficient duration on an 8-hour schedule o dosage to produce the anticipated hypotensive effect. Since hypotension ha been produced in man by intravenous administration of the harmala alkaloids,[7, 9] a gross correlation still exists between MAO inhibition and hypotensio with this group of compounds.

Comment

Unfortunately, any statement concerning the mechanism by which drug such as JB-516, iproniazid, and other MAO inhibitors lower blood pressure i still speculative. The hypotension produced by JB-516 in man resembles tha seen after surgical sympathectomy. Because of species variation in vascula response, it is not entirely valid to use the usual pharmacological procedure in dogs to establish the site of action, whether central or peripheral. It should be kept in mind that MAO is involved in the metabolism of several biogeni amines, all of which have cardiovascular activity. It will probably be impossible to explain various actions of MAO inhibitors on the basis of changes i the metabolism of only 1 or even 2 of these amines. It seems paradoxical tha hypotension could be due to blocking the metabolism of pressor amines. I is of interest in this connection that some patients with norepinephrine-producing tumors (pheochromocytomas) may have supine *hyper*tension but marke postural *hypo*tension. Hypotensive episodes also occur in patients with hyperserotonemia due to malignant carcinoid.

Regardless of what the precise mechanism of action of these drugs on bloo pressure may be, continued study of MAO inhibitors in patients with hypertension is warranted and suggests a new approach to the mechanism of this disease

Acknowledgment

Walter Lovenberg and Consuelo Muellenberg performed most of the chemica procedures used in this study. H. H. Orvis and I. G. Tamagna, George Washington University School of Medicine, Washington, D. C., cooperated in som of the studies on iproniazid. We are indebted to The Upjohn Co., Kalamazo Mich., for supplying serotonin capsules; to Hoffmann-La Roche Inc., Nutley N. J., for the iproniazid; to Lakeside Laboratories, Inc., Milwaukee, Wis., fo the JB-516; and to Chas. Pfizer & Co., Inc., Brooklyn, N. Y., for the harmaline

References

1. SJOERDSMA, A., L. GILLESPIE & S. UDENFRIEND. 1958. A simple method for the measurement of monoamine oxidase inhibition in man. Lancet. **273:** 159.
2. MASTER, A. M. 1958. Iproniazid (Marsilid) in angina pectoris. Am. Heart J. **56:** 57
3. NUSSBAUM, H. E., W. LEFF, V. D. MATTIA & E. HILLMAN. 1957. The effects of iproniazid phosphate (Marsilid) on hypertension. Angiology. **8:** 198.

4. Harnes, J. 1958. The effect of Marsilid on blood pressure in hypertensive patients. J. Clin. Exptl. Psychopathol. **19:** (2) Suppl. 1, 152.
5. Weissbach, H., B. Redfield & S. Udenfriend. 1958. Serotonin-O-glucuronide; an alternate route of serotonin metabolism. Federation Proc. **17:** 377.
6. Gillespie, L., L. L. Terry & A. Sjoerdsma. 1958. A new anti-hypertensive agent, 1-phenyl-2-hydrazinopropane. Presented at Meeting of Am. Heart Assoc. San Francisco, Calif.
7. Gunn, J. A. 1935. Relations between chemical constitution, pharmacological actions and therapeutic uses, in the harmine group of alkaloids. Arch. intern. pharmacodynamie. **50:** 379.
8. Udenfriend, S., B. Witkop, B. G. Redfield & H. Weissbach. 1958. Studies with reversible inhibitors of monoamine oxidase. Harmaline and related compounds. Biochem. Pharmacol. In press.
9. Pennes, H. H. & P. H. Hoch. 1957. Psychotomimetics, chemical and theoretical considerations; harmine, Win-2299 and Nalline. Am. J. Psychiat. **113:** 887.

John A. Oates and Perola Zaltzman (*Section on Experimental Therapeutics, National Heart Institute, Public Health Service, Bethesda, Md.*): The development of an alternate method of measuring monoamine oxidase (MAO) inhibition in man has emerged from recent work in our laboratory on the indole amine tryptamine.

In 1937 Werle first reported decarboxylation of tryptophan in animal tissues.[1] It was only recently that this observation was confirmed in our laboratory. In addition, tryptamine has now been demonstrated in mammalian brain, liver, and kidney. A marked rise in tissue concentrations was found following MAO blockade with iproniazid. Tryptamine is metabolized primarily via monoamine oxidase to indoleacetic acid. Although other minor metabolic pathways may be available to tryptamine, studies to date suggest that it is more dependent on the MAO pathway than are such amines as serotonin and norepinephrine. This lack of a major alternate pathway of breakdown produces striking accumulation of tryptamine when MAO is inhibited, leading to its increased urinary excretion. The development of a specific and sensitive method for measuring tryptamine in the urine has provided a tool for studying this and other aspects of its metabolism in man.

In normal subjects, the urinary excretion of tryptamine was found to be in the range of 30 to 125 μg./day. However, in 6 patients to whom 25 mg./day of the MAO inhibitor 1-phenyl-2-hydrazinopropane (JB-516) was administered, sixfold to tenfold increases in urinary tryptamine have occurred consistently. A typical example is seen in figure 1, from which the definite relationship between dosage of JB-516 and urinary tryptamine is apparent. On the currently recommended dose of iproniazid (50 mg./day), a slight but definite increase (twofold to threefold) in urinary tryptamine was observed, this being less than the increment produced by 12.5 mg. of JB-516. After the drug was discontinued, excretion of the amine diminished progressively, but clear evidence of inhibition was present even after 7 days.

From these observations it appears that the assay of urinary tryptamine affords a simple measure of MAO inhibition in man that seems to be a general reflection of its effect throughout the body. With this index of inhibition, either alone or in conjunction with the serotonin metabolism test, information can be gained regarding the degree of MAO inhibition on a given regimen, as well as the rapidity of its onset and duration of effect. These tests bring the assay of enzyme inhibition out of the *in vitro* realm into the area of clinical

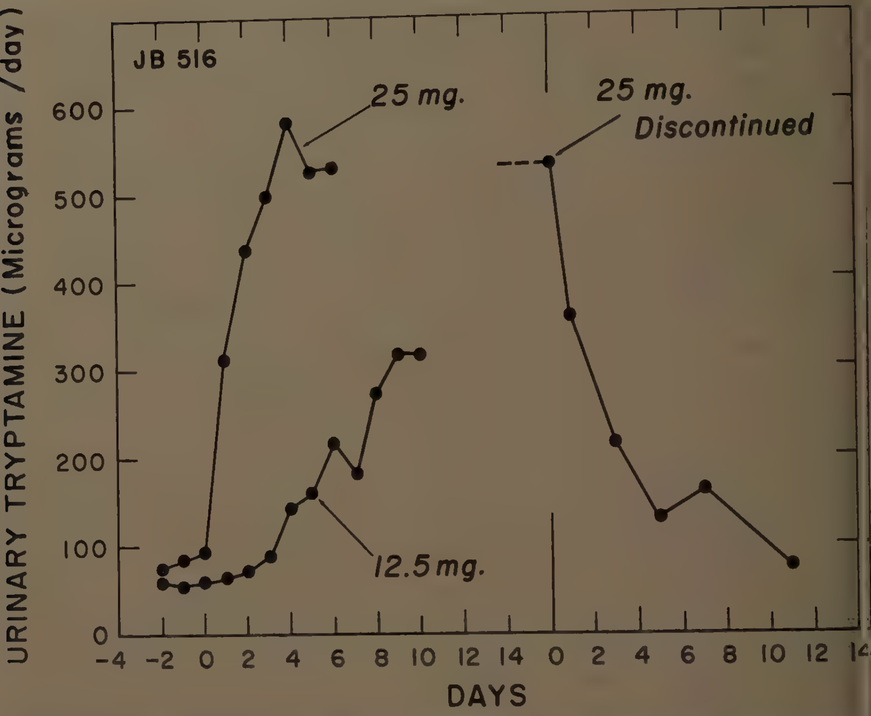

FIGURE 1. Effect of JB-516 on urinary tryptamine. The time scale on the abscissa is divided into 2 sections, that on the left representing days following initiation of drug therapy, and that on the right representing the number of days following discontinuation of the drug.

studies, where both biochemical and pharmacological effects may be observed in parallel.

Reference

WERLE, E. & C. MENNICKEN. 1937. Über die Bildung von Tryptamin und von Tyramin aus Tyrosin durch tieresches Gewebe. Biochim. Z. **291:** 325.

DALE G. FRIEND (*Peter Bent Brigham Hospital, Boston Mass.*): Sjoerdsma and his collaborators have presented a new, intriguing, and very instructive approach to the problem of the treatment of hypertension, utilizing an established biological system for its presumed mechanism of action. The principle of administering serotonin or its precursor and measuring the resultant metabolite with and without monoamine oxidase inhibition should prove to be a most useful procedure in ascertaining the monoamine oxidase blocking ability of an agent. It is interesting to note that ephedrine does not show monoamine oxidase-blocking activity when tested in this manner, a fact that supports an opinion that many of us have held for some time.

It would be interesting to know how hypertensive patients on iproniazid (Marsilid) or other monoamine oxidase inhibitors would respond if they were given large amounts of serotonin precursors over long periods. In our experience with iproniazid and with another exceedingly potent monoamine oxidase inhibitor, given alone, neither has been satisfactory for the treatment of hypertension. Although a hypotensive effect occurs in most patients, it is for the most part of the orthostatic type, and large doses of iproniazid often are required to obtain this effect.

Since we know that monoamine oxidase inhibition followed by norepinephrine infusion does not influence blood pressure, we do not believe that the norepinephrine system is involved in this effect. In our experience with normal individuals whose monoamine oxidase had been blocked with iproniazid in a dose of 3 mg./kg. of body weight for 3 days prior to an infusion of 600 μg. of norepinephrine, there was no difference in blood pressure values before and after the administration of iproniazid. It is interesting to speculate on the role of normetanephrine in such a system. Axelrod[1] has shown that in the presence of monoamine oxidase block, norepinephrine is mainly converted to normetanephrine through O-methylation. Consequently, blocking the monoamine oxidase system would not be expected to influence markedly the metabolism of norepinephrine, since this system is readily available. I have observed this reaction in normals. However, the question arises: does the normetanephrine thus produced, although nearly inactive itself, serve as an interfering substance by blocking some of the effect of norepinephrine, as could be accomplished by occupying receptor sites? Apparently this is not the case, for when a normal patient is subjected to monoamine oxidase block with iproniazid and the system flooded with norepinephrine (analogous to serotonin flooding), no effect is observed on the blood pressure.

Other aspects of this problem are worthy of consideration. We must not forget the role of agents on sodium metabolism in arterial walls and certainly in the kidney. For example, it has been recently shown in animals that, following injection into the renal artery of phenoxybenzamine (Dibenzylene), the sympathetic peripheral blocking agent, with resultant block of sympathetic nerve activity to that kidney, there occurs a large outpouring of sodium, that continues for some time. Other agents that block the release of norepinephrine apparently do likewise. As yet we do not know how such agents influence the sodium content in the vessel walls. Certainly, sodium is a most important factor in sensitizing the vessel walls to sympathetic amines and, as is well known, is intimately related to the problem of hypertension. It may be that monoamine oxidase inhibition through interference with the metabolism of amines other than norepinephrine can block sympathetic action in some way and result in a release of sodium from the kidney and perhaps from arterial walls. This effect would certainly lead to a fall in the blood pressure. It is worth trying to flood the blocked monoamine oxidase system with agents that in themselves may have little effect, but that may have surprising properties when not metabolized by monoamine oxidase. Several agents in this category may be readily considered.

Finally, I offer a word of caution. I have been impressed by the few cases of severe hepatic necrosis that I have seen in patients receiving iproniazid, and I feel that the molecule does have sufficient inherent toxic properties to cause concern if it is to be given to unselected patients in large doses for long periods. Although many patients have been on iproniazid for many months, I do not know of any reaction similar to that seen after hydralazine hydrochloride such as in the disseminated lupus erythematosus picture. We must be on the aler for this possibility, since iproniazid and 1-phenyl-2-propylhydrazine (JB-516 are hydrazines. Long-continued use of hydrazines in such a chronic diseas as hypertension must be followed closely in view of past experience with com pounds of this type.

There are exceedingly potent monoamine oxidase inhibitors that are no hydrazines. We have had experience with one of these that has activity of th same order as 1-phenyl-2-propylhydrazine (JB-516), but that did not give satisfactory effect in the treatment of patients with depression. Furthermore it did not exhibit marked hypotensive properties.

Finally, it should be remembered that agents such as G-22355 (Tofranil which, however, is not a monoamine oxidase inhibitor, have been shown to hav a beneficial effect in depressed patients in Europe[2] and, to a limited extent, in the United States also.

References

1. Axelrod, J. 1957. O-methylation of epinephrine and other catechols *in vitro* and *i vivo*. Science. **126:** 400.
2. Kuhn, R. 1957. Über Die Behandlung depressiver Zustande mit linem iminodibenzyl derivat (G-22355). Schweiz. Med. Wochschr. **87:** 1135–1140.

E. A. Zeller (*Northwestern University Medical School, Chicago, Ill.*): It i shown in table 1 of this paper that iproniazid is fairly ineffective as an inhibito of monoamine oxidase of rat liver homogenate, having a pI_{50}-value slightl above 3. This is in accordance with data which we published in 1955.[1] Mito chondrial monoamine oxidase, however, is much more sensitive to iproniazi (Marsilid). A considerable amount of iproniazid seems to be bound to som cytoplasmic components. Thus the typical action of iproniazid is almost los in liver homogenate, and I wonder why the authors did not use mitochondri as an enzyme source.

Reference

1. Zeller, E. A., J. Barsky & E. R. Berman. 1955. Amine oxidases. XI. Inhibition o monoamine oxidase by 1-isonicotinyl-2-isopropylhyrazine. J. Biol. Chem. **214:** 267,

A. Sjoerdsma: The use of crude homogenates is adequate for purposes o comparing one compound with another.

EFFECT OF IPRONIAZID ON SURVIVAL IN THE DOG, FOLLOWING ACUTE CORONARY OCCLUSION*

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Introduction

After acute occlusion of a major coronary artery in the conscious dog, circulatory failure is associated with the onset of ventricular fibrillation. Experimental evidence has shown that, in dogs, the onset of ventricular fibrillation following coronary occlusion may be related to sensory stimulation of sympathetic afferents superimposed upon the myocardial damage[1-4] (TABLES 1 and 2).

Ventricular fibrillation in man frequently follows acute coronary occlusion or coronary insufficiency. Cesarman,[5] Cossio,[6] Schweizer,[7] and Master[8] found that iproniazid§ relieved the pain of coronary insufficiency in patients. This suggested to us that iproniazid might prevent the onset of ventricular fibrillation following acute coronary occlusion in the conscious dog.

Material and Methods

Mongrel dogs weighing 10 to 20 kg. were used. One week prior to the experiment, with the animal under pentobarbital anesthesia, a ligature was placed loosely around the anterior descending branch of the left coronary artery at its junction with the circumflex coronary artery (FIGURE 1). Loose ends were carried bilaterally through the chest wall, and the chest was closed. One week later, without anesthesia, occlusion was produced by tightening the ligature through the chest wall. Continuous femoral arterial blood pressure (B.P.) and single-channel electrocardiographic (EKG) tracings were recorded prior to, during, and after coronary occlusion. The femoral arterial catheter was inserted under local procaine-ephedrine anesthesia, and the blood pressure was recorded through a mercury manometer. At death or sacrifice the heart was examined for gross and histological changes.

Two groups of animals were used: untreated controls and iproniazid-treated animals, the latter divided into 3 subgroups: (1) those receiving iproniazid intramuscularly (I.M.) for 4 days prior to coronary occlusion; (2) those receiving iproniazid intramuscularly (I.M.) 30 min. prior to acute occlusion; and (3) those receiving iproniazid intravenously (I.V.) immediately following acute occlusion.

Drug dosage was 2 or 2.5 mg./kg. (I.M.) of iproniazid. Doses of 3.5 mg./kg. have been given to dogs for 13 weeks with apparently no ill effects.[9]

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† Department of Medicine.

‡ Department of Surgery.

§ Marsilid, product of Hoffmann-La Roche Inc.

Table 1

Survival in Dogs Following Acute Coronary Occlusion

	Number	Death in 1 hour	Total percentages dead	Total percentages survived
Conscious control	12	8	66.6	33.3
Manning, McEachern, and Hall (1940, low tie)	22	9	40	60
Unilateral sympathectomy (left) McEachern	8	3	37.5	62.5
Bilateral sympathectomy McEachern	12	0	0	100

Table 2

Deaths from Ligature Around Anterior Descending Ramus*

One-Stage Procedure Under Nembutal

	Number	Death in 24 hours	
		No.	Percentages
Control	26	16	62
Quinidine	26	12	46
Staged bilateral thoracic ganglionectomy	28	11	39
Procaine stellate ganglion injection immediately following infarction	50	8	16

* Reproduced by permission of the *American Heart Journal* (Milch *et al.*[3]).

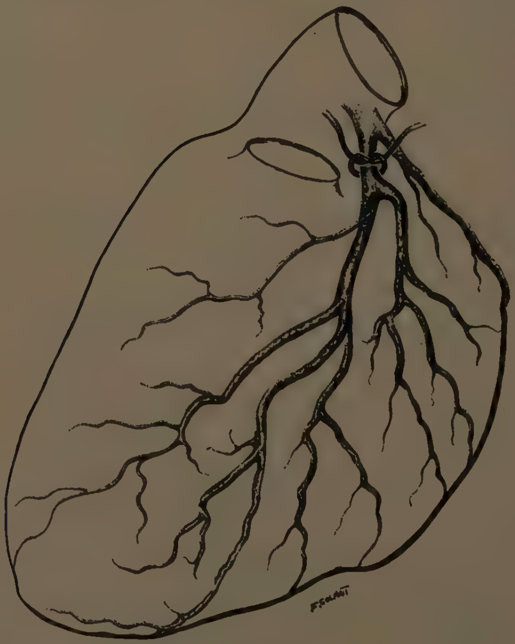

Figure 1

Results

Untreated control group. Within 5 min. following occlusion, ventricular fibrillation and death took place in 7 of 12 of the untreated dogs (58.3 per cent: TABLE 3). All animals that developed fibrillation died, while those in which fibrillation did not develop survived. No major drop in B.P. was observed until the onset of ventricular fibrillation (FIGURE 2 shows the correlation between fibrillation and the drop in the B.P.). The range of onset of fibrillation was from 1 min. 10 sec. to 10 min. 40 sec. following occlusion.

TABLE 3

EFFECT OF IPRONIAZID* ON SURVIVAL FOLLOWING ACUTE CORONARY OCCLUSION

	Number	Death within 1 hour	Additional deaths within 24 hours	Percentage of acute survival beyond 1 hour post-occlusion	Survived beyond 24 hours	Percentage of survival beyond 24 hours
Conscious controls	12	8	1	33.3	3	25
Iproniazid treated	14	3	4	78.6	7	50

* In doses of 2 to 2.5 mg./kg. of body weight given I.M. to healthy, 10- to 20-kg. mongrel dogs for 4 days prior to acute coronary occlusion.

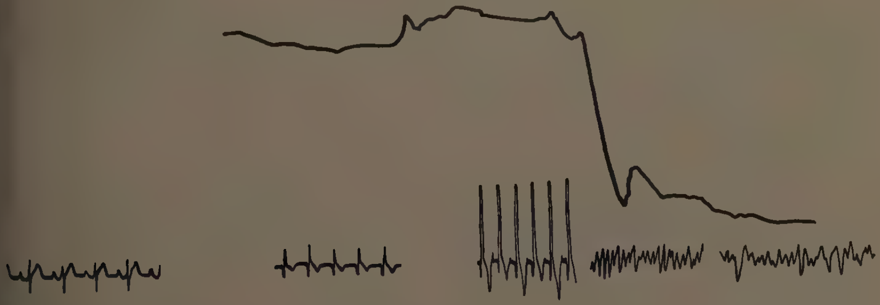

FIGURE 2. Onset of death from ventricular fibrillation in 8 dogs. Range is 1 min. 10 sec. to 10 min. 40 sec., with an average of 3 min. 8 sec.

After tightening of the ligature through the chest wall there was an acute rise in blood pressure followed by a return to control values or by a transient fall. The B.P. remained elevated above 90 mm. Hg until the onset of ventricular fibrillation or a ventricular tachycardia that changed within seconds to ventricular fibrillation.

Chronic iproniazid-treated (4-day) group. Fourteen dogs received iproniazid (I.M.) for 4 days prior to occlusion: five received 2.5 mg./kg./day and 9 received 2.0 mg./kg./day. On the fourth day of drug administration, 2 to 3 hours following the last dose of iproniazid, acute coronary occlusion was produced.

Three dogs died acutely with ventricular fibrillation at 1 min. 10 sec., 5 min. 30 sec., and 8 min. Failure to fibrillate within 1 hour was considered acute survival from the occlusion. Eleven of 14 dogs (78.6 per cent) survived the acute episode. Four dogs died 1 to 24 hours after occlusion. Thus, survival beyond 24 hours was 50 per cent, and 7 of 14 animals survived beyond 1 week and were sacrificed. There was no death beyond 24 hours postocclusion.

There was no preocclusion EKG pattern consistent with an iproniazid effect, nor any postocclusion pattern of ischemia that seemed different from the control. No appreciable difference was noted in the appearance of EKGs other than the absence of fibrillation in animals that survived, whether iproniazid-treated or not. Blood pressure changes did not differ in the two groups.

Chronic iproniazid-treated, preocclusion group. Two dogs receiving 2.5 mg./kg. of iproniazid I.M. 30 min. prior to acute coronary occlusion survived without ventricular fibrillation. The EKG changes observed in these dogs did not differ from those of the control or the chronically treated group. There was no acute rise in blood pressure following occlusion in these animals, which was a result different from that found with the control group or the animals treated with iproniazid for 4 days prior to occlusion.

Chronic iproniazid treated, postocclusion. Iproniazid (2.5 mg./kg.) given intravenously immediately after coronary occlusion will be reported on at a later date.

Discussion

Iproniazid administered for 4 days prior to the onset of acute coronary occlusion prevents death in dogs by protecting them from the rapid onset of ventricular fibrillation. In this respect iproniazid is not unique, since pentobarbital anesthesia,[10, 11] morphine sulfate, reserpine,[11] and hexamethonium[12] also seem to protect against the onset of ventricular fibrillation (TABLE 4). Chlorpromazine administered intravenously immediately following acute coronary occlusion also protects conscious dogs against ventricular fibrillation if they receive ether anesthesia 30 to 45 min. before placement of the femoral arterial catheter[11] (TABLE 5). In addition to these agents that affect the conscious state of the animal, bilateral thoracic sympathectomy[1] (TABLE 1) and procaine stellate ganglion block[3] in a 1-stage procedure (TABLE 2) prevent the onset of ventricular fibrillation.

It appears from these data that an afferent stimulus (for example, pain) superimposed upon myocardial damage may alter the resistance of the heart to ventricular fibrillation. Anesthesia, analgesia, tranquilizers, ganglionic blocking agents, and iproniazid all seem to protect against the onset of ventricular fibrillation. It is necessary to stress the fact that these drugs are prophylactic because, once ventricular fibrillation begins, following acute coronary occlusion, these agents do not reverse the process. Unlike quinidine or the newer basic steroids,[31] which are also antifibrillatory, these drugs, except for hexamethonium,[12, 31] produce no obvious change in the EKG pattern.

Ganglionic blocking. In a fashion similar to that of ganglionic blocking agents, iproniazid produces orthostatic hypotension, dryness of the mouth, impotence, difficulty in micturition, and decreased intestinal motility.[13] In our experience, hexamethonium prevents the onset of ventricular fibrillation in dogs (TABLE 4). The mechanism of action may relate to alterations in pain perception, as seen in the clinical success with tetraethylammonium chloride

(TEAC), which prevents anginal and pleuritic pain.[14] TEAC has been used clinically in the treatment of coronary insufficiency, with results and side effects similar to those of iproniazid.[15] Iproniazid or some product of its metabolism may affect ganglionic transmission in man.

TABLE 4
SURVIVAL IN DOGS FOLLOWING ACUTE CORONARY OCCLUSION

	Number	Acute death (within 1 hour)		Acute survival (within 1 hour)		Total dead in 24 hours		Total survival beyond 24 hours	
		No.	Percentages	No.	Percentages	No	Percentages	No.	Percentages
Conscious control	12	8	66.6	4	33.3	9	75	3	25
Iproniazid, 2–2.5 mg./kg., I.M., for 4 days prior to occlusion	14	3	21.4	11	78.6	7	50	7	50
Reserpine, 25 μg./kg., I.M., 2½–3 hours prior to occlusion	10	2	20.0	8	80.0	3	30	7	70
Hexamethonium, 0.5 μg.–5.0 μg./kg., I.V., prior to occlusion	16	6	37.5	10	62.5	8	50	8	50
Pentobarbital anesthesia, I.V., 25 mg./kg. prior to occlusion	6	0	0	6	100	0	0	6	100
Morphine sulfate, I.M., 8–10 mg./kg. 30 min. prior to occlusion	4	0	0	4	100	0	0	4	100

TABLE 5
SURVIVAL IN DOGS AFTER ACUTE CORONARY OCCLUSION OF ANTERIOR DESCENDING RAMUS OF LEFT CORONARY ARTERY

	Number	Death in 1 hour	Death in 24 hours	Total percentages dead	Total percentages survived
Conscious control	12	8	1	75	25
Chlorpromazine, 2 mg./kg., I.V., immediate post occlusion (30–45 min. postether anesthesia)	7	1	1	28.5	71.5
Chlorpromazine, 2 mg./kg., I.V., immediate postocclusion (procaine-ephedrine, local)	4	4	0	100	0
Chlorpromazine, 10 mg./kg., I.V., immediate postocclusion	2	1	0	50	50

Adrenergic blockade by iproniazid. Greisemer *et al.*[16] showed that iproniazid blocked the response to adrenaline of the cat nictitating membrane and rabbit aorta. Although Koelle[17] showed this effect to be a biphasic one, the action of iproniazid as an adrenergic blocking agent may be a major factor in preventing the onset of ventricular fibrillation. This action would be similar to that of dihydroergotamine which, as shown by Manning and Caldwell,[4] using a technique similar to ours in the conscious dog, prevents the onset of ventricular fibrillation.

The role of serotonin, adrenaline, and noradrenaline in relation to iproniazid's monoamine oxidase–inhibiting effect. The inhibition of monoamine oxidase[18] and the possible action of iproniazid as it relates to these compounds at ganglionic and effector sites has been presented in an excellent schema by Koelle[19] and by Marrazzi.[20] The alternative metabolic pathways such as *o*-methylation[21] and how these relate to the myocardium have not been ascertained.

Iproniazid causes a rise in the catecholamine content of the heart.[22] The increase in catecholamines may not be deleterious despite Raab's evidence[23] to the contrary. We have data that show that noradrenaline given prior to or immediately following acute coronary occlusion does not predispose to ventricular fibrillation, and may actually be protective against the onset of ventricular fibrillation in the conscious dog (TABLE 6). This effect may be a function of the dose and timing of administration of noradrenaline, as suggested by Hoffman and his associates,[24] who showed a biphasic effect of adrenaline and noradrenaline in its effect on vulnerability of the myocardium to fibrillation. The factor of ventricular irritability that predisposes to ventricular fibrillation in "adrenaline syncope" is perhaps not parallel to what occurs clinically after acute or chronic coronary occlusion in man. Certainly, our data in the dog (TABLE 6), showing possible protection from noradrenaline, would support this. Bulle[25] has shown that serotonin injected locally in the myocardium produces a marked ischemia. Systemic and local effects can be quite dissimilar. The key to survival in the dogs after acute coronary occlusion may be the rapid development of collateral circulation to the blocked area. There is evidence that iproniazid increases coronary blood flow,[26] as does serotonin (Crumpton *et al.*[27]) and, logically, this could increase collateral circulation.

TABLE 6

SURVIVAL IN DOGS AFTER ACUTE CORONARY OCCLUSION OF ANTERIOR DESCENDING RAMUS OF LEFT CORONARY ARTERY

	Number	Death in 1 hour	Death in 24 hours	Total percentages dead	Total percentages survived
Conscious control	12	8	1	75	25
Noradrenaline, 4 mg./1000 cc. 5% G/W gtts. 20/min., simultaneous with occlusion	3	1	0	33.3	66.6
Noradrenaline, 0.2–0.4 mg. I.V., immediate postinfarct followed by gtts. 20/min. 4 μg./cc.	3	1	0	33.3	66.6

Direct effect on the myocardium. Apart from its effect in increasing coronary blood flow, iproniazid produced a negative inotropic effect on the isolated perfused cat heart.[20] This is also true of hydroxyzine (Vistaril, Atarax), which Hutcheon[28] has shown decreases the adrenaline-induced automatic activity of papillary muscles. Hydroxyzine possesses a potent antiarrhythmic effect on the heart. It would be of interest to see if iproniazid has similar effects on the isolated papillary muscle. Brooks and his associates[29] state that "antifibrillatory drugs stabilize the membrane in that they tend to prevent changes in transmembrane potential by applied stimuli but do not modify either resting or action potentials." Activity of this kind for iproniazid on heart muscle has not yet been reported, but that it can affect another tissue in this way may be seen in its prevention of seizures following electroconvulsive stimuli.[30]

Summary and Conclusions

Iproniazid given prior to acute coronary occlusion prevented the onset of the ventricular fibrillation and death that follows rapid occlusion of a major coronary artery in the conscious dog. In this effect it resembles a diverse group of compounds that includes reserpine, pentobarbital, morphine sulfate, and hexamethonium.

References

1. McEachern, C. G., G. W. Manning & G. E. Hall. 1940. A.M.A. Arch. Internal Med. **65:** 661.
2. Manning, G. W., C. G. McEachern & G. E. Hall. 1939. A.M.A. Arch. Internal Med. **64:** 661.
3. Milch, E., W. T. Zimdahl, R. W. Eagen, T. W. Hsia, A. A. Anderson & D. Joseph. 1955. Am. Heart J. **50:** 483.
4. Manning, G. W. & G. C. Caldwell. 1947. Brit. Heart J. **9:** 85.
5. Cesarman, T. 1957. Arch. inst. cardiol. Méx. **27:** 563.
6. Cossio, P. 1957. La Prensa Med. Argentina. **44:** 2679.
7. Schweizer, W. 1958. Cardiologia. **33:** 40.
8. Master, A. M. 1958. Am. Heart J. **56:** 570.
9. Benson, W. M., P. L. Stefko & M. D. Roe. 1952. Am. Rev. Tuberc. **65:** 376.
10. Olichney, M. & W. Modell. 1958. Federation Proc. **17:** 401.
11. Hoffmeister, F. S., W. Regelson & H. Rubin. 1958. Surg. Forum. **9:** 242.
12. Regelson, W., F. S. Hoffmeister & H. Wilkens. Work in progress.
13. Cesarman, T. 1958. J. Clin. Exptl. Psychopathol. **19:** Supp. 1, 159.
14. Israel, H. L., E. L. Keeter, F. E. Urbach & W. D. Willis. 1949. New Engl. J. Med. **241:** 738.
15. Hirschleifer, I., G. Schwartz, H. J. Fuerst, A. Fankhauser. 1952. N. Y. State J. Med. **52:** 575.
16. Griesemer, E. C., C. A. Dragstedt, J. A. Wells & E. A. Zeller. 1955. Experentia. **11:** 182.
17. Kamijo, K., G. B. Koelle, H. H. Wagner. 1955. J. Pharmacol. Exptl. Therap. **117:** 213.
18. Zeller, E. A., J. Barsky, J. R. Fouts, W. F. Kircheimer & L. S. Van Orden. 1952. Experentia. **8:** 349.
19. Koelle, G. B. 1958. J. Clin. Exptl. Psychopathol. **19:** Supp. 1, 37.
20. Marazzi, A. 1958. *Ibid.* **19:** Supp. 1, 44.
21. Axelrod, J. 1957. Science. **126:** 400.
22. Pletscher, A. 1958. Experentia. **14:** 73.
23. Raab, W. 1953. Cardiologia. **22:** 291.
24. Hoffman, B. F., A. A. Seibens, P. F. Cranefield & C. McC. Brooks. 1955. Circulation Research. **3:** 140.
25. Bulle, P. H. 1957. Science. **126:** 24.
26. Pletscher, A. & B. Pellmont. 1958. J. Clin. Exptl. Psychopathol. **19:** Suppl. 1, 163.
27. Crumpton, C. W., C. A. Castillo, C. G. Rowe & G. M. Maxwell. 1958. Serotonin and the dynamics of the heart. Amine Oxidase Inhibitors. Ann. N. Y. Acad. Sci. **80**(3)**:** 960.
28. Hutcheon, E. E., A. Scriabine & D. L. Morris. 1956. J. Pharmacol. Exptl. Therap. **118:** 451.
29. Brooks, C. Mc. C, B. F. Hoffman, E. E. Suckling & O. Orias. 1955. Excitability of the Heart. Grune and Stratton. New York, N. Y. & London, England.
30. Prockop, D., P. A. Shore & B. B. Brodie. 1958. Anticonvulsive properties of monoamine oxidase inhibitors. N. Y. Acad. Sci. **80**(3)**:** 643.
31. Schallek, W., F. W. Zabransky, L. M. Jampolsky, W. R. Rehl & M. W. Goldberg. 1957. Proc. Soc. Exptl. Biol. Med. **95:** 433.

IPRONIAZID IN CARDIOVASCULAR THERAPY, WITH SPECIAL REFERENCE TO ITS ACTION IN ANGINA PECTORIS AND BLOOD PRESSURE

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The clinical effects of iproniazid (IPN, or Marsilid*) in man can be grouped according to the four following functions: (1) stimulation of the central nervous system, (2) anabolism, (3) autonomic equilibrium, and (4) cytotoxicity (hepatitis).

These effects were known before my colleagues and I reported the effect of this medication on the clinical picture of angina pectoris.[1] Since then, we have been able to confirm all of the effects reported in the literature on the pain aspect of angina, on some vascular complaints such as Raynaud's disease, and other peripheral circulatory insufficiencies, including arteriosclerosis obliterans and endarteritis obliterans. By analyzing these effects from the clinical point of view, we have tried to determine how the drug acts. The study of the effects of IPN on angina pectoris and on blood pressure are presented below.

Material and Method of Work

One hundred and ninety-one patients with angina pectoris of various grades of severity were studied (TABLE 1). The patients were divided into four groups in accordance with the intensity of the angina pain in relation to exertion. Patients classed as Grade 4 presented characteristic angina pain upon any exertion, as well as during rest and clinostatism. Grade 3 included those who had pain with the exertion involved in dressing and walking slowly for distances up to 20 meters, but who had no pain during rest or in clinostatism. Grade 2 patients suffered pain from great and moderate exertion, which was considered walking 100 meters at a normal pace or up to 50 meters at a rapid pace. Finally, Grade 1 included those patients who experienced angina pain only upon running, rapid walking, or stair climbing. Due to its wide variability, pain linked to emotional disturbances or temperature changes was not taken into account in the classification. A characteristic of all the cases was that pain could be controlled by rest or nitroglycerin, which was generally needed for Groups 3 and 4. In the majority of Group 2 cases, suspension of effort was sufficient to produce disappearance of pain, while in Group 1 rest alone was sufficient. Accordingly, 72 patients were classed in Group 4, 39 in Group 3, 34 in Group 2 and 46 in Group 1.

All patients received the usual treatment for angina except for radioiodine (I^{131}) and surgical revascularization. IPN was administered when the symptoms persisted in spite of previous treatment. Electrocardiographic studies at rest were carried out prior to as well as during treatment with IPN and on discontinuance and on readministration of the drug. The exercise-tolerance test was carried out on a small number of patients.

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* Hoffmann-La Roche.

Of the 191 patients, 108 were male and 83 female, and their ages ranged from 28 to 77 years, with the distribution as shown in FIGURES 1 and 2. The 28-year-old patient was a case of rheumatic endomyocarditis with angina who had undergone surgery for mammary ligation.

TABLE 1
CLASSIFICATION OF SEVERITY OF PAIN OF ANGINA

Grade	No. of cases	G	Angina pain		C
			M	S	
4	72	+	+	+	+
3	39	+	+	+	
2	34	+	+		
1	46	+			
Total	191	191	145	111	72

(G) Great efforts; (M) Medium efforts; (S) Small effort; (C) Clinostatism.

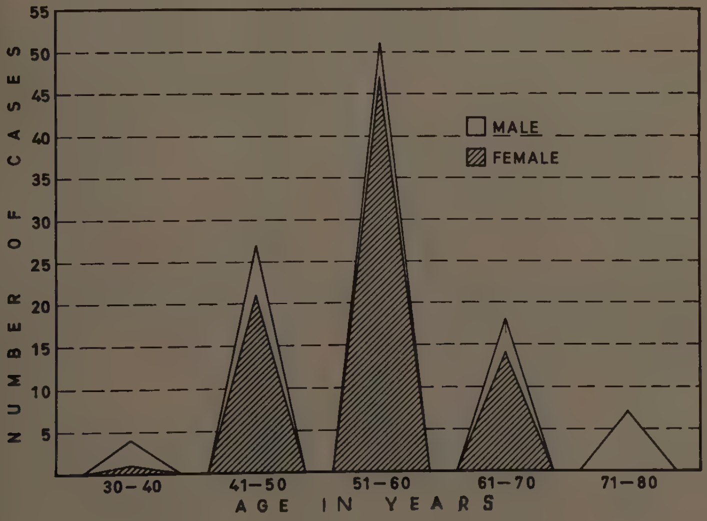

FIGURE 1. Distribution of cases by sex and age.

Essential hypertensives comprised 34.5 per cent; normotensives, 53.3 per cent; systolic hypertensives, 12 per cent; diabetics, 20.4 per cent; luetics, 12 per cent; rheumatic heart disease, 1 case; coarctation of the aorta, 1 case.

The history of angina in the 191 patients varied from 2 months to 10 years, with an average of 3½ years; in 37 per cent it was 1 to 2 years, in 48 per cent longer than 2 years, and in the remaining 15 per cent it was 2 to 11 months.

Once the patients were classified according to severity of their angina pain and their electrocardiographic tracing, and their response to previous treatment was verified, IPN was administered at the rate of 150 mg./day in 3 divided doses. With the exception of those receiving treatment for cardiac insufficiency or diabetes, this was the only treatment. Studies were made of the course of the angina pain during treatment, the consumption of nitroglycerin, exertion capacity, and response to orthostatism. Analyses were made of the response of the angina pain to great, moderate, and slight exertion, as well as in clinostatism. Side effects, their time of appearance and disappearance, intensity, and development were analyzed. The recurrence of angina pain after discontinuing the treatment and the time of its disappearance or improvement on renewing the medication were studied. Analysis was made of the conditions calling for discontinuance of treatment, appearance of infarction and its course during treatment, frequency of pain, and exacerbation of symptomatology and, where it could be determined, the cause of death during treatment. In order to study the duration of action, treatment was suspended for 3 weeks in 20 cases; at the end of this period it was renewed in 17 of the patients. Also considered were the course of angina pain in cases with severe hypotension secondary to medication, habituation, and effects on cardiac frequency, arrhythmia, the general state of the patient, cardiac failure, and other pains.

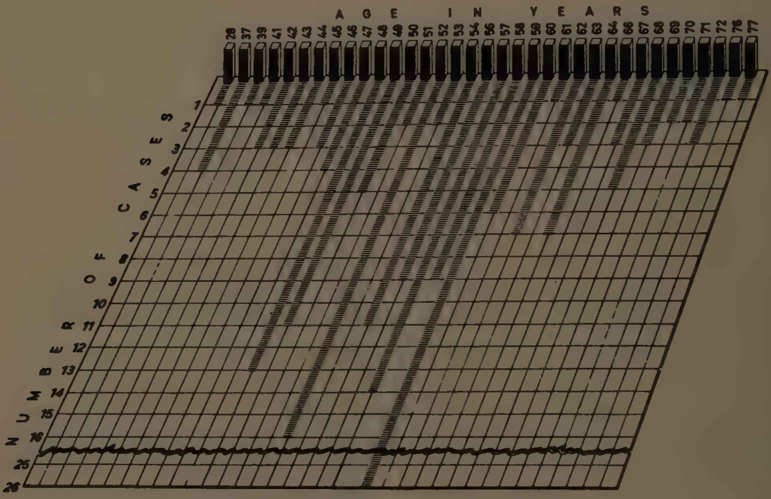

FIGURE 2. Distribution of cases by age.

To establish the dosage for initial and supporting treatment, we began with 150 mg./day, as mentioned above. When total disappearance of pain on moderate exertion was obtained, the dosage was reduced to 100 mg. and to 75 mg. day. Where pain reappeared, the dosage was restored to 150 mg. and, in a few cases, increased to 200 mg. Another reason for decreasing the dosage was the appearance of side effects, which were so severe in some cases as to mask the effects on the angina symptoms.

Blood pressure was taken in clinostatism and orthostatism in all patients each time they were seen. The average pretreatment blood pressure is herein referred to as "initial figure." The response was calculated as percentage of reduction in systolic and diastolic pressures from this initial figure, both during clinostatism and orthostatism. Blood pressure, with age and the percentage of hypotension after medication, was correlated with intensity of side effects. The percentage of reduction in blood pressure in normotensive, essential hypertensive, and systolic hypertensive patients was calculated for each group both in clinostatism and in orthostatism. Ophthalmoscopic examinations were too few in number, and the duration of the study was too short to permit definitive conclusions.

Laboratory studies included complete blood count (CBC) examinations, liver-function, blood chemistry, suprarenal function, and thyroid tests. With the exception of the CBC studies, the small number of laboratory examinations did not warrant any conclusions.

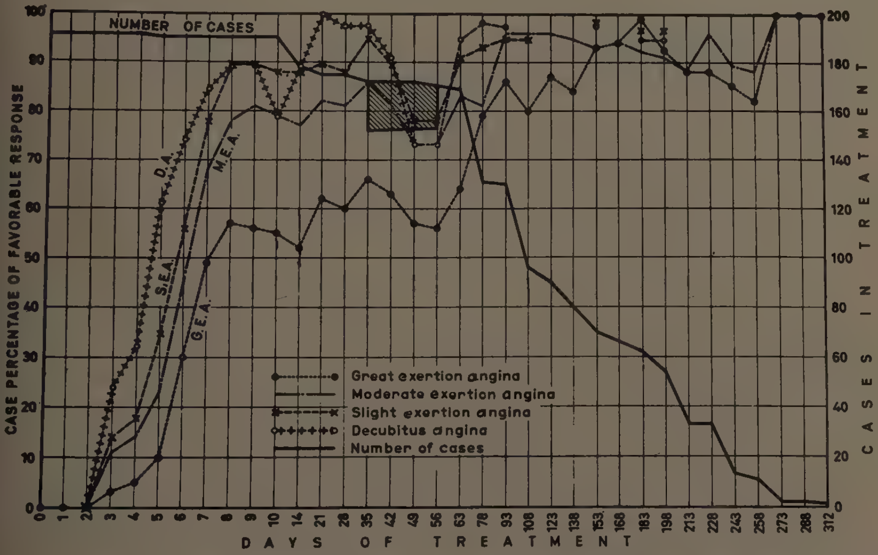

FIGURE 3. The evolution of angina pectoris in relation to days of treatment.

Effects on Angina

Angina pain in clinostatism. This condition was present in 72 of the patients studied. The first positive response to therapy was observed in 24 per cent of the patients on the third day of treatment. Improvement increased to 84 per cent on the seventh day and to 97.4 per cent on the thirty-fifth day of treatment. At this point the medication was withdrawn from 20 patients. Improvement diminished to 90.8 per cent in 7 days, to 74 per cent in 14 days, and to 73 per cent in 21 days. This result indicated that the symptomatology reappears between 1 and 2 weeks after withdrawal of the drug. After 7 days of restored medication there was again improvement in 94.8 per cent of the cases, indicating that the disappearance of pain by readministration is more rapid than reappearance of symptoms through withdrawal. As may be seen from FIGURE 3, improvement continued after the second month in patients in whom it had not been necessary to withdraw medication because of side effects.

Angina pain with slight exertion. This condition was observed in 111 of the patients. The first response to the drug occurred in 14 per cent of the patients on the third day of treatment, the number increasing to 90 per cent on the eighth day. This improvement was maintained in 94.8 per cent of patients treated for 35 days; medication was then withdrawn from 20 patients. Improvement diminished to 78 per cent in 14 days, indicating that this symptom reappears between 1 and 2 weeks after suspension of medication. On renewing it, improvement had increased to 91.4 per cent at the end of 7 days, and was observed in 96 per cent of those patients who continued treatment for 5 months or more.

Angina with moderate exertion. This condition occurred in 145 of the patients. Improvement occurred in 11 per cent of the patients on the third day, in 81 per cent on the ninth day, and was maintained until the thirty-fifth day. On withdrawing the medication from 20 patients, improvement diminished to 73 per cent after the fourteenth day, returning to 83 per cent in 7 days after the reinstitution of medication. The patients who continued treatment for 90 days showed an improvement of 96 per cent, which was maintained with only slight variations.

TABLE 2
RÉSUMÉ OF COURSE OF ANGINA PECTORIS UNDER TREATMENT:
CLASSIFICATION BY DEGREE OF PAIN

Duration in days	7	14	28	60	90	120
	Percentages					
Pain with great exertion	49	52	60	64	86	87
Pain with moderate exertion	68	77	81	83	96	96
Pain with slight exertion	78	88	88	91.4	95	94.9
Pain in clinostatism	84.3	89.5	97.4	94.8	98	98
Cases under treatment	190	177	175	169	130	90

Angina pain with great exertion. This condition was observed in all the patients studied. The first response was noted in 3.2 per cent of the cases on the third day, in one half the patients in a week, and in 66 per cent on the thirty-fifth day. On withdrawing the medication from 20 patients, improvement diminished to 63 per cent within 1 week, to 57 per cent in 2 weeks, and to 56 per cent in 3 weeks. On reinstitution of treatment recuperation of 64 per cent was noted. Three months later a response was noted in 85 per cent of the cases.

TABLE 2 summarizes the effects of iproniazid on angina pain in the entire group of patients, regardless of severity. If the disappearance of pain resulting from great exertion is taken as the base level of response, it can be said that at the end of the first week of treatment response had been obtained in 49 per cent of the cases; at the end of the second week the degree of improvement had hardly risen (52 per cent) and, at the end of the first month, 60 per cent of the patients reported improvement, a rate that was maintained until the end of the second month of treatment. At the end of the third month the improvement increased to 86 per cent of the cases, and was maintained at that level throughout treatment.

Taking as a response base the disappearance of angina pain on moderate exertion at the end of the first week, improvement was obtained in 68 per cent of the cases; it rose to 96.1 per cent at the end of the third week, and thereafter was maintained throughout treatment.

The disappearance of angina pain on slight exertion and in clinostatism followed a parallel course, with a rapid response of 78 per cent and 84.3 per cent, respectively, after the seventh day; at the end of the first month the response for pain in clinostatism was 97.4 per cent and, for pain with slight exertion, it was 88 per cent. At the end of the second month a response was obtained in more than 90 per cent of the cases.

As seen from the results summarized in TABLE 3, patients of Group 4 responded better, and improvement declined in the other 3 groups. At the end of the first week 55.7 per cent of the patients of Group 4 reported total or almost complete disappearance of symptomatology and alleviation that increased progressively until the third month of treatment; by that time 75 per cent of the patients had responded favorably. The remaining 25 per cent of the patients did not respond significantly or at all.

TABLE 3

EVOLUTION OF ANGINA PECTORIS UNDER TREATMENT: CLASSIFICATION BY GRADE OF SEVERITY

Days	7	14	28	60	90	120
Groups						
1	39.2	36.4	36.0	32.0	34.4	25.8
2	46.8	51.6	56.4	58.8	72.8	72.8
3	52.0	57.8	61.0	63.5	73.8	73.8
4	55.7	61.2	65.2	66.6	75.0	75.0

In Group 3, 52 per cent of the patients reported total or almost complete disappearance of symptomatology at the end of the first week. This percentage increased progressively to 73.8 per cent of the cases by the third month. The remaining cases did not respond.

In 46.8 per cent of the cases of Group 2 favorable response was observed on the seventh day, increasing progressively until the third month, when improvement was obtained in 72.8 per cent, while the degree of improvement in the remaining patients was similar to that of the other groups. Finally, in Group 1 there was improvement in 39.2 per cent, and this percentage decreased progressively so that by the fourth month only 25.8 per cent had responded favorably. In 74.2 per cent of the cases there was no favorable response, or medication was suspended because of side effects.

Pain During Treatment

Pain was observed in 28 per cent of the patients who had responded favorably to treatment, but who occasionally presented pains of variable intensity and duration, often repeatedly during several days. In 4 of these cases there was evidence of an infarction. In some cases the dosage was raised to 200 mg. in 24 hours and decreased after the disappearance of symptoms. In other cases the dosage was maintained in spite of decreased pain. Medication was suspended in the cases of infarction. Another interesting fact is that in 9.4 per cent (18 cases) exacerbation of the angina symptomatology was observed in the first week, which induced the suspension of medication. However, in subsequent cases the pain decreased after this phase of exacerbation.

Infarction During Treatment

During treatment, 7 instances of infarction without the classic symptomatology occurred. Three of them were totally asymptomatic and were discovered on routine electrocardiographic examination; in the remainder, infarctions occurred with appearance of isolated, intense pains of short duration that disappeared spontaneously or with the administration of nitroglycerin. In the remaining 4 patients the pains occurred during rest, in one of them during the day, and in the other 3 at night; in these the pain was sufficient to cause arousal. The pains did not recur. None of these cases of myocardial infarction belonged to the group of severe hypotensives described below.

These infarctions occurred in 2 patients with angina of recent origin and in 5 others with angina of long standing.

Response Time

The first responses were observed after the third day and up to the thirty-fifth day, with a maximum response in some cases between the fourth and seventh days. The time of appearance of first responses was variable for the different grades of angina pain; that is, more rapid in clinostatism, a little slower for pain with slight exertion, still slower on moderate exertion and, finally, with great exertion, the response, although first observed on the third day, appeared only in 50 per cent of the cases on the seventh day.

A marked change from one grade of severity of angina pain to a lesser one, such as from Grade 4 to Grade 2, was observed from the fourth day of treatment, but with greater frequency from the fourteenth day.

Total responses, that is, those in which angina symptomatology disappears completely, were also first seen on the fourth day in a few cases, frequently after the first month of treatment, and more frequently after the third month of treatment. This response interval is shortened when treatment is renewed after discontinuance.

Dosage

The dosage based on body weight averaged 2.5 mg./kg./day, with a maximum of 3.4 mg./kg./day and a minimum of 1.7 mg./kg./day, but no relation between dosage and response was noted.

Effects of Suspension

In 84 per cent of the patients who had a favorable response, with cessation of pain, symptoms returned between 7 and 14 days after withdrawal although, in a large number of cases, these did not recur until 1 month later, while in 16 per cent anginal symptoms have not recurred after suspension, the longest asymptomatic lapse being 5 months.

Angina and Arterial Hypotension

In hypertensive and normotensive subjects no increase of angina symptomatology was observed either in asymptomatic hypotension or during symptomatic hypotensive crises, with dizziness, lipothymia, and fainting. Nevertheless, in electrocardiographic studies made during the hypotensive state or hypotensive crises, increased ischemia was noted in many cases. Hypotension was a frequent cause for withdrawal of the medication, more in normotensives than in hypertensives, indicating that the hypertensive angina case is a better subject for treatment than is the normotensive.

Cardiac Failure

Twenty-three patients presented pretreatment cardiac failure; 2 of them died. Fourteen had suffered myocardial infarctions: 3 diaphragmatic, 8 anteroseptal, and 3 diaphragmatic and anteroseptal infarctions. Seven patients, all hypertensive, had neither clinical nor electrocardiographic histories in infarction, 2 were neither hypertensive nor had histories of myocardial infarction, and 1 was diabetic. During treatment no modification of the picture of myocardial failure was observed that could be attributed to IPN, nor were digitalic or diuretic requirements modified.

TABLE 4
DEATHS OBSERVED DURING TREATMENT

No. of Cases	Cause	Severity of angina pain	Response to treatment	Type of death
7	?	4	Excellent	Sudden
1	Painless infarction with collapse	4	Excellent	Collapse
1	Hepatitis	4	Excellent	Hepatic coma

Deaths During Treatment

Nine deaths were observed during treatment, 7 of them sudden, without the clinical picture of infarction (that is, there was neither pain nor collapse), 1 with collapse lasting 14 hours, but without pain, and 1 who died in hepatic coma (TABLE 4). The 7 patients who died suddenly had received the medication for a period longer than 1 month, and all of them had responded very favorably. The patient who died in collapse had taken the medication for one week and had been a severe angina case with a high consumption of nitroglycerin and had also required the occasional use of morphine during nocturnal anginal pain crises. He had responded favorably and rapidly to the medication prior to collapse and death. The patient who died in hepatic coma had received the drug for 3 months, but it had been withdrawn 10 days before the appearance of jaundice.

Effect on Blood Pressure

If we take the 19 patients as a group, independently of whether they were hypertensive, normotensive, or hypotensive, we observe that the pressure under the influence of IPN follows the course shown in FIGURE 4.

Clinostatism. In 42 per cent of the cases there was no modification of systolic blood pressure in clinostatism, while in 54.1 per cent there was a decrease from 0.1 to 30 per cent from the pretreatment levels (FIGURE 4A). In the greatest percentage of cases systolic arterial tension in clinostatism was reduced between 5.1 and 15 per cent from the initial figures.

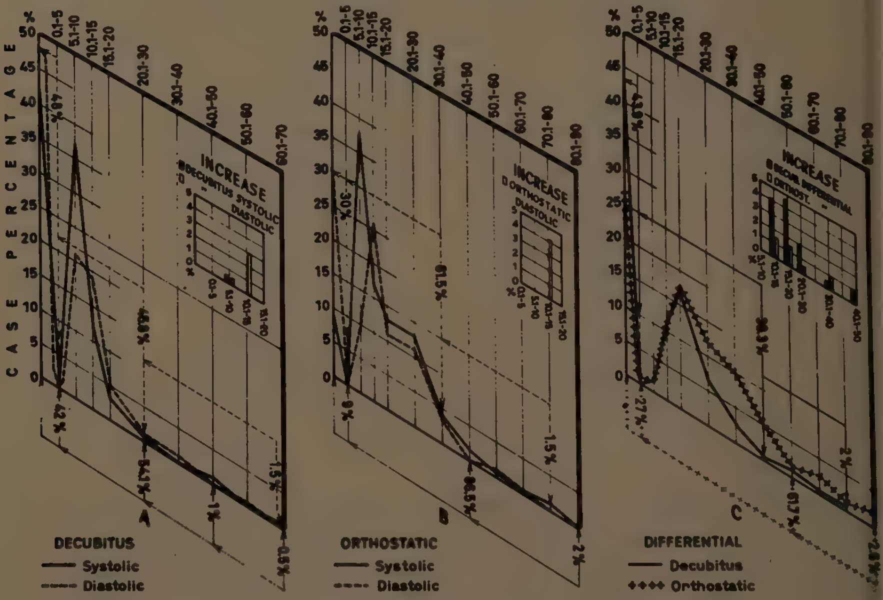

FIGURE 4. Reduction in the percentage of blood pressure.

In 48 per cent of the cases there was no reduction of the diastolic blood pressure on clinostatism, and in 45.8 per cent there was a pressure drop of between 0.1 and 30 per cent (FIGURE 4A).

Summarizing the observations, blood pressure (systolic and diastolic) was modified in clinostatism in approximately 50 per cent of the cases. The systolic pressure was modified in a slightly larger number of cases than the diastolic and, except for a few cases, in the majority the pressure drop was 5 to 15 per cent off the initial pressure, a figure not significant in patients under any treatment.

Orthostatism. In 9 per cent of the cases there was no modification of the systolic pressure, while in 86.5 per cent a drop of 0.1 and 50 per cent of the pretreatment pressure values was observed. In 4 cases the pressure drop was greater than 50 per cent (FIGURE 4B).

In 30 per cent of the cases there was no reduction of diastolic pressure in orthostatism, while in 61.5 per cent the observed blood pressure drop varied between 0.1 and 40 per cent of the pretreatment pressure values; in 2 cases the drop was greater than 50 per cent (FIGURE 4B). It may be seen, therefore, that both systolic and diastolic pressures are modified in orthostatism, but that the former is modified in a greater percentage of cases (91 per cent) than the latter (70 per cent). Systolic and diastolic blood pressures are modified in a

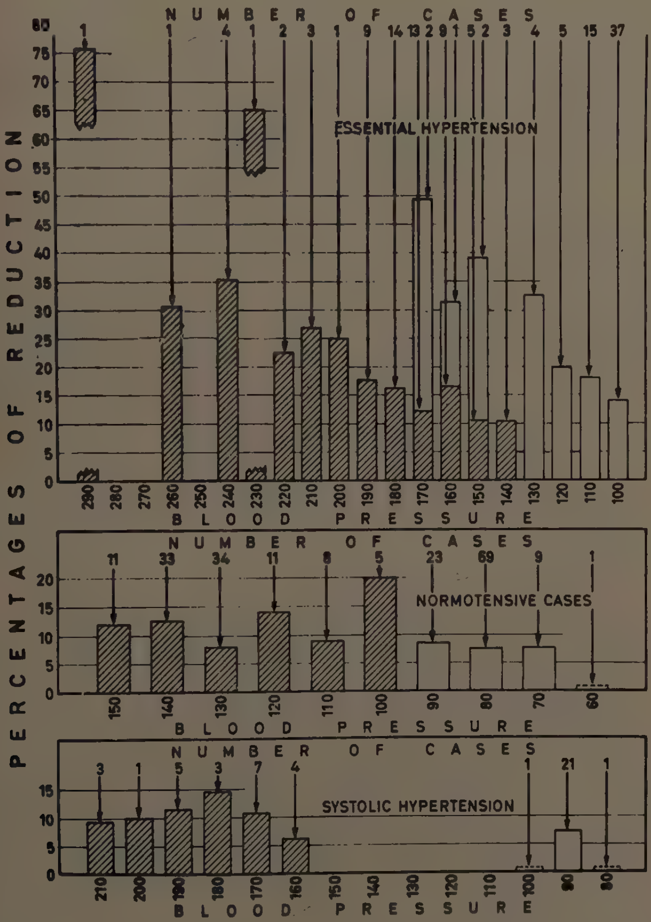

FIGURE 5. Reduction of orthostatic systolic pressure readings (shaded bars) and diastolic pressure readings (open bars) in relation to initial figures.

significant percentage of cases between 5.1 and 30 per cent of the pretreatment figures. We believe that this drop can be attributed to the pharmacological action of IPN and, when compared with the modification of the pressure in clinostatism, its action resembles that of the ganglionic blocking agents.

If we divide the patients studied into 3 groups: essential hypertensives, systolic hypertensives, and normotensives (FIGURE 5), we observe that hypotension was more important and significant in the essential hypertension group as regards both systolic and diastolic pressures. In the normotensive group

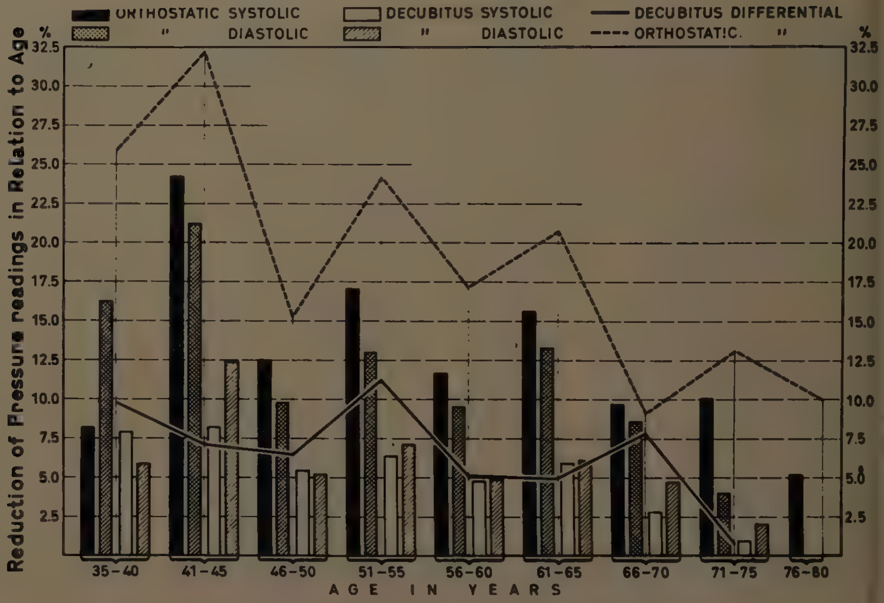

FIGURE 6. Differential blood pressure in clinostatism.

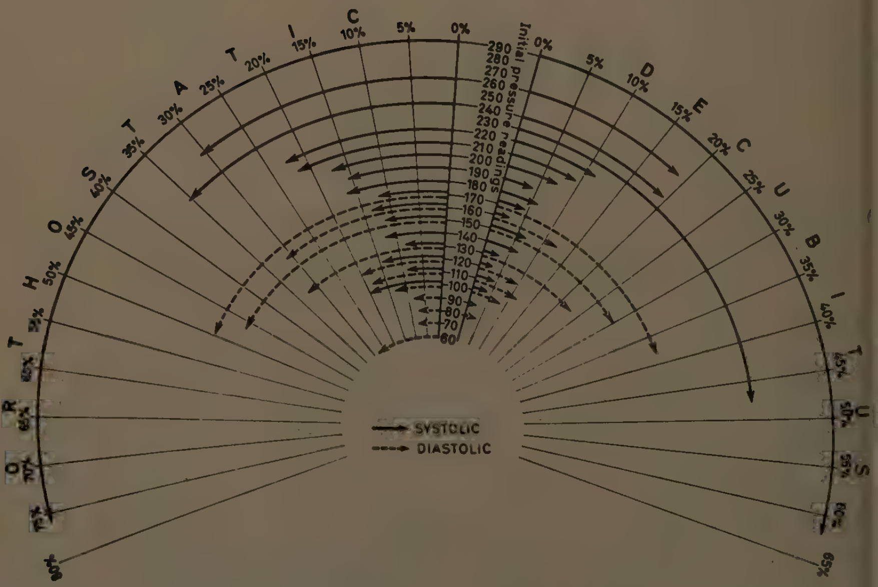

FIGURE 7. Reduction of blood pressure readings in relation to initial figures.

a lowering of pressure was in some cases of little significance; in others it was significant. In systolic hypertensives pressure was lowered, but only slightly and insignificantly.

If we correlate the reduction of blood pressure with age, it is seen that, the younger the patient, the greater the decrease in both systolic and diastolic pressures. The differential blood pressure in clinostatism undergoes great variation in the young subjects, and these variations become less and less important with increasing age (FIGURE 6).

If we correlate the drop of blood pressure in relation to the initial figures, the greater the initial figure, both systolic and diastolic, the greater the percentage of reduction in blood pressure (FIGURE 7).

The hypotensive response parallels the anginal syndrome in onset, duration, and course, which might suggest a common mechanism.

Comment

We have noted in a preliminary report that IPN prevents the appearance of angina pains and improves the capacity for exertion. Since this accidental discovery 18 months prior to this report, the results have been corroborated, and the frequency and importance of the side effects have been studied. These observations have enabled us to understand the manifestations and, by their analysis, to glimpse something of the pharmacological mechanism of action. As discussed below, there are important reasons for considering IPN as a ganglioplegic agent; the blockade of the afferent fibers that conduct heart pain to the conscious centers could constitute the very essence of the action on the pain of angina pectoris. This concept would also explain the occurrence of "silent" infarctions in the course of treatment.

If we compare the effects of IPN with those of atropine, dibenzyline, tetraethylammonium tetrachloride (TEA), hexamethonium salts (C_6), pentolinium, and mecamylamine, we observe that the atropinic, muscarinic, and ganglion-blocking pharmacological actions of IPN become apparent (TABLE 5).

When comparing speed of action, duration, elimination of the drug and the disappearance of its effects, it is of interest to study the routes of administration, as well as the dosage employed.

IPN is absorbed rapidly by the intestine, a fact that makes the oral route useful. Studies of blood concentration with multiple doses have not yet been made, but the duration of the clinical effects suggests accumulation, the therapeutic concentration being sustained for as long as 4 weeks after suspension of medication.

We can say of IPN that the degree of blockage is not predictable since, as may be seen in FIGURES 1–9, various degrees of blockage were obtained in different patients with the same dosage. TEA administered intravenously or intramuscularly acts very rapidly and, if sustained effects are desired, it is necessary to repeat the dosage frequently or to administer it by venoclysis.

Since C_6 is eliminated more slowly, its orthostatic hypotensor effect is more lasting than its effect in clinostatism, and in some cases habituation is produced. It acts as rapidly as TEA, depending on the methods of administration, but its effects are more prolonged.

With IPN the clinical effects are slow to appear; in anginal pain they begin to be observed on the third day, increase progressively with treatment, and disappear slowly on suspension of the drug. A form of potentiation is also observed since, on renewal of the medication, the return of the first responses occurs earlier. The intensity and duration of action appear to depend more on the duration of treatment than on the dosage employed, probably because of cumulative effects. The more prolonged the treatment with constant dosages, the more intense the action (FIGURE 3, TABLES 2 and 3).

TABLE 5

COMPARATIVE TABLE OF THE PHARMACOLOGICAL ACTIONS OF CHOLINERGIC, ADRENERGIC, GANGLIONIC BLOCKING AGENTS, AND IPN

System, organ, or function	Atropine	Dibenzyline*	TEA C6 Pentolinium Mecamylamine	IPN
Pupillary sphincter	Mydriasis Cycloplegia Impairment of accommodation Blurred vision	Miosis	Mydriasis Impairment of accommodation Blurred vision Ptosis	Mydriasis Impairment of accommodation Blurred vision
Cardiac rate	Tachycardia	Bradycardia	Tachycardia	Bradycardia and tachycardia
Cardiac output	Diminution		Diminution	Increase?
Rhythm	Atrioventricular block			
Coronary arteries				Vasodilatation
Skin and mucous membrane	Vasodilatation		Vasodilatation	Vasodilatation
Blood pressure		Orthostatic hypotension	Orthostatic hypotension	Orthostatic hypotension
Digestive secretions	Dry mouth and diminished secretions in general		Dry mouth and diminished secretions in general	Dry mouth
Sphincters		Relaxation		
Tone and motility	Diminution		Diminution	Diminution
Urinary tract tone	Diminution		Diminution (urinary hesitancy)	Diminution (urinary hesitancy)
Bladder sphincter	Contraction			Contraction
Sweat glands	Anhydrosis		Anhydrosis	Diaphoresis
Lacrimal glands	Secretion inhibited			
Orgasm				Delayed
Erection	Diminution?	Increase?	Incapacity	Incapacity

* Phenoxybenzamine hydrochloride.

An interesting fact, based on the first 100 cases, is the parallelism between the magnitude of hypotensive action of the drug and the severity of the collateral symptoms that results from adrenergic and cholinergic blocking; this fact strengthens the hypothesis that IPN acts as a ganglioplegic agent (FIGURES 8 & 9). The side effects of iproniazid are listed in TABLE 6.

FIGURES 8 and 9 represent hypotension, divided into 8 groups according to

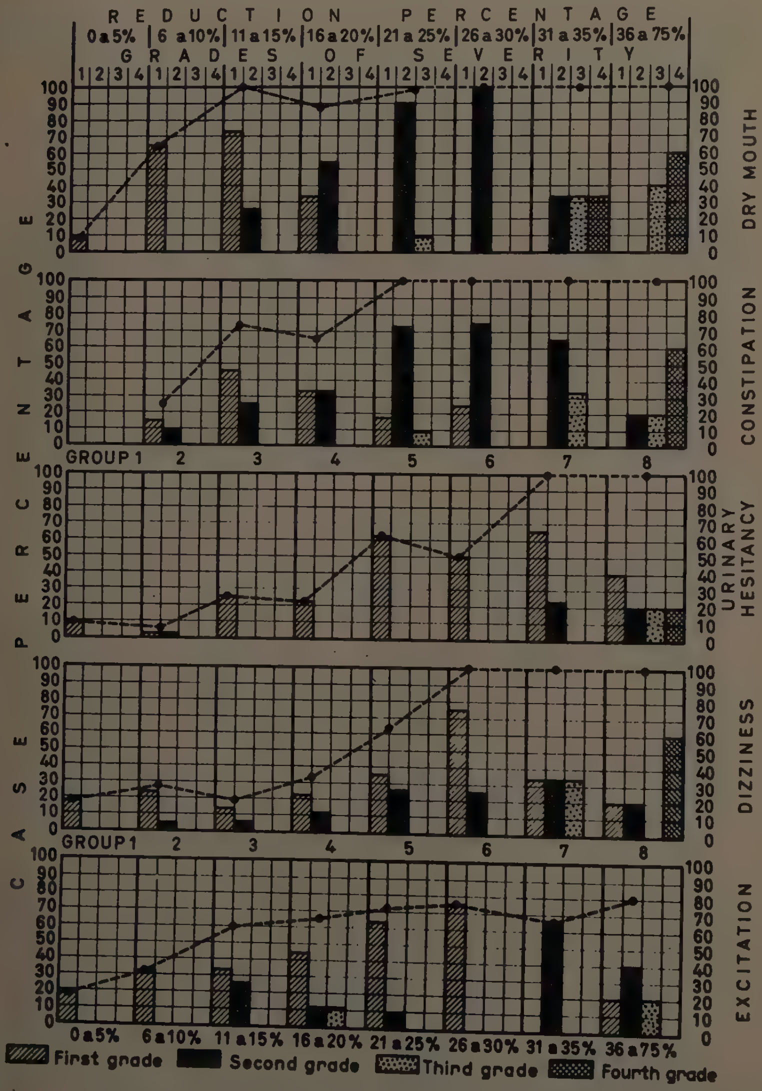

FIGURE 8. The significance of collateral symptoms as compared to the reduction of orthostatic systolic blood pressure.

degree. Correlating the grade of arterial hypotension with the frequency and severity of the side effects, we observe the following:

Dryness of the mouth is observed in Groups 1 and 2, in which there is little or no arterial hypotension. This dryness increases rapidly with increased hypotension, and is present in almost all patients who have a drop in orthostatic systolic pressure greater than 10 per cent. It may be considered that this symptom appears at first independently of hypotension, but later follows a parallel course both in frequency and severity of both symptoms.

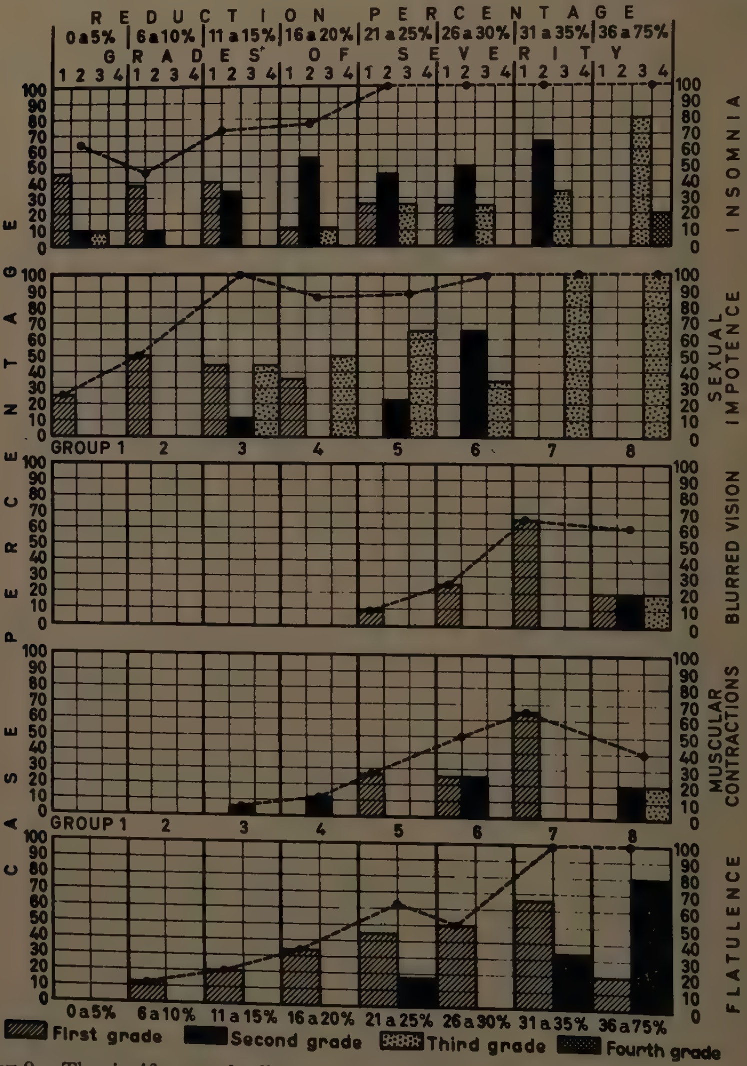

FIGURE 9. The significance of collateral symptoms as compared to the reduction of orthostatic systolic blood pressure.

Meteorism begins to be observed in Group 2 and becomes progressively more

TABLE 6

SIDE EFFECTS OF IPRONIAZID, ACCORDING TO THEIR SEVERITY

	Dryness of Mouth
Grade 1.	Sensation of dry mouth, not disturbing to patient.
Grade 2.	Disturbing sensation that patient tolerates.
Grade 3.	Disturbing sensation that patient tolerates with difficulty.
Grade 4.	Intolerable sensation; buccal pharynx dry, bitter taste.
	Flatulence
Grade 1.	Increased elimination of gases by mouth and rectum.
Grade 2.	Abdomen distended and tense.
	Constipation
Grade 1.	Loss of regularity of bowel movements.
Grade 2.	Marked decrease in the number of bowel movements, but with response to mild laxatives.
Grade 3.	Marked reduction in bowel movements; strong laxatives required.
Grade 4.	Total obstruction, simulating ileus paralyticus.
	Difficulty in micturition
Grade 1.	Slight delay noticed by patient when alerted to look for it.
Grade 2.	Slight delay in micturition noticed by patient spontaneously.
Grade 3.	Disturbing delay.
Grade 4.	Inability to urinate, necessitating bladder catheterization.
	Impotence
Grade 1.	Delay in orgasm.
Grade 2.	Delay in orgasm followed by inability of erection.
Grade 3.	Total inability of erection, not preceded by delay in orgasm.
	Blurring of vision
Grade 1.	Difficulty in accommodation, but not disturbing.
Grade 2.	Disturbing difficulty in accommodation.
Grade 3.	Great difficulty in accommodation, with pupillary paresia.
	Dizziness
Grade 1.	Infrequent but not disturbing sensation.
Grade 2.	Frequent disturbing sensation of dizziness.
Grade 3.	Severe dizziness, with vertigo but without falling.
Grade 4.	Dizziness, vertigo, sometimes with falls, with and without fleeting loss of consciousness.
	Twitching and/or muscular tremors
Grade 1.	Twitching of large muscle masses, diurnal and nocturnal, but controllable when on guard.
Grade 2.	Twitching controlled voluntarily with difficulty, but not continuous.
Grade 3.	Not voluntarily controllable.
	Excitation
Grade 1.	Euphoria and only slight increase in activity.
Grade 2.	Euphoria with apparent increase in activity, not disturbing.
Grade 3.	Great euphoria and tendency to activity of manic type, but responsive to medication.
Grade 4.	Mania, euphoria, and hyperactivity persisting in spite of medication.
	Insomnia
Grade 1.	Delay in getting to sleep, not disturbing.
Grade 2.	Disturbing delay in getting to sleep; early awakening.
Grade 3.	Delay in getting to sleep and early awakening, responding with difficulty to normal dosages of sedatives and barbiturates.
Grade 4.	Total inability to sleep, which disappears when the drug is suspended.

pronounced in frequency and intensity as the blood pressure drop increases. It is found in all cases in which the pressure drop is greater than 30 per cent of the initial figures.

Constipation begins to be observed in Group 2, increases rapidly in frequency and intensity as blood pressure drop becomes more pronounced, and is found in all cases in which the pressure drop is greater than 20 per cent. This progression in intensity of the symptom would appear to indicate a common mechanism.

Difficulty in micturition, like dryness of the mouth, is observed particularly in male patients, beginning with Groups 1 and 2, in whom the blood pressure drop is not significant. Its frequency increases slowly, but the intensity does not change until Group 7, when the pressure drop is more than 30 per cent and is seen in a severe degree in every patient. Since it was not known that the symptom had been produced by the pharmacological action of IPN, a prostatic resection was done on a Grade-4 patient in Group 8.

Sexual impotence and delay in ejaculation are observed in groups 1 and 2 in a significant percentage of cases, independently of the hypotensive effect. Beginning with Group 3, a marked increase in frequency and intensity is observed, and the resulting curve follows a marked parallelism with that observed in dryness of the mouth. Beginning with Group 3, practically all patients present the symptom and, as the percentage of hypotension increases, impotence or lack of erection also becomes greater in intensity.

The blurring of vision is a difficult symptom to study. While possibly unnoticed in the lower grades, difficulty in accommodation was observed beginning with Group 5. The symptom was shown by a maximum of 66 per cent of patients in Group 7, Grade 1; in Group 8 it increased in intensity, and 3 of the 5 patients in this group presented iridoparalysis. Since it is observed only in the cases with significant hypotension, that is, more frequent and intense in Groups 7 and 8, we can conclude that there is a common mechanism.

Dizziness is first observed infrequently in Groups 1, 2, 3, and 4, increasing in frequency in Groups 5 and 6, and in all patients in Groups 7 and 8 who show a marked increase in intensity. It thus appears that in many patients the dizziness is not related to the drop in blood pressure, but dizziness secondary to the orthostatic hypotension effect appears when the pressure drop is 20 per cent or more, which would explain its increase in frequency and intensity. It may be concluded that dizziness has two etiologies, one probably central and the other secondary to the orthostatic hypotension.

Twitching and muscular tremors, which were not observed in patients of Groups 1 and 2, began to appear in a mild form in Group 3, and increased slightly in frequency and intensity in the rest of the groups. In spite of this increase, a clear parallelism is not observed.

Excitation begins to be observed in Groups 1 and 2, increasing slightly and progressively in frequency and intensity in Groups 3 and 4, diminishing in intensity in Groups 5 and 6, and increasing again in Groups 7 and 8. While it increases in frequency as hypotension increases, the course of intensity is not the same.

Insomnia appears in Groups 1, 2, 3, and 4, with significant frequency and intensity and, even when it increases in frequency to include all the remaining groups, it is encountered with significant frequency in the cases in which hypotension is not important. This effect would indicate an independence of mechanisms.

Discussion

It is known that the cervical sympathetic nerves transmit painful impulses to the central nervous system; the attempts to divide these nerves surgically or block them chemically with the object of suppressing anginal pain were based on this concept. These procedures affect neither the pain nor its cause, but intercept the conductor pathways, thus preventing the passage of the painful impulses. A fundamental argument begins with the premise that coronary pain follows the nerve pathways until it reaches the central nervous system, resulting in consciousness of pain. Nevertheless, the hypothesis has been presented that coronary pain occurs without intervention of nerve pathways, but rather through chemical substances or phenomena of electric induction. Even if this possibility cannot be absolutely eliminated, the constancy of localization of coronary pain and its radiations suggest that it follows the anatomical nerve pathways.

The characteristics of IPN as an adrenergic and cholinergic blocking agent indicate it particularly for the suppression of coronary pain. The characteristics of IPN are the intensity of its action, its better tolerance compared with other ganglioplegic agents, and its prolonged cumulative effect. We therefore conclude that this is the mechanism of action of IPN in the suppression of anginal pain. The favorable electrocardiographic modifications found in some cases could be explained on the basis of decrease in vasoconstrictor tone of the coronary vessels with increased coronary flow and cardiac output. In summary, it may be said that the ganglioplegic effect of IPN can explain the suppression of coronary pain in the chronic as well as in the acute patient and when the intensity of response depends on the intensity of blockade. This last conjecture is confirmed in clinical observation, since the disappearance of coronary pain is significant when the collateral effects of the adrenergic blockade are more intense. Due to the slowness of its action (2 or 3 days for first responses with the usual dosage), IPN would appear to have no effect on the acute pain of the patient without pretreatment when it is administered orally. The time of action when it is used parenterally is not known.

The hypotensor effect of IPN may be considered from two points of view: as a complication in the treatment of angina or as a useful therapeutic agent in hypertensive patients. Concerning the first of these, no severe coronary complication resulting from hypotension during treatment were observed, not even in those cases in which the pressure drop was considerable or even with fainting in orthostatism. An increase of ischemia was observed in some electrocardiographic tracings, but in none was infarction a consequence of the hypotension. Notwithstanding, we can say that the drop in the pressure is a potential risk, requiring continuous vigilance of patients under treatment and, at any given moment, suspension of IPN administration.

Concerning the second point, namely, the utilization of its hypotensive action in the treatment of hypertensive patients, the results with these 191 patients permit the statement that it is a potent hypotensor with a more important action in orthostatism, but also in clinostatism, even though to a lesser grade and in fewer patients. This hypotensor effect may be observed in hypertensives as well as in normotensives, but the intensity of hypotensor action of IPN is not predictable.

The characteristics that we have attributed to IPN as an adrenergic blocking agent in angina pectoris make it a particularly good medication for the treatment of high blood pressure. Its intense, prolonged action permits, better than do rapidly eliminated blocking agents, the maintenance of a more constant level of pressure. This prolonged effect can be detrimental, since in some cases severe hypotension prevented the patients from taking an orthostatic position for as long as 4 weeks. Compression bandaging of the lower extremities and of the abdomen were insufficient to control the orthostatic hypotension and forced the patients into clinostatism until the pharmacological effect had disappeared sufficiently.

It is of interest that the pressure was lowered in patients who had not otherwise responded or who had not tolerated other blocking agents.

In summary, IPN could be a good hypotensor whose utility is subject to patient tolerance of side effects that are important in cases of effective hypotension (FIGURES 8 and 9).

The most feared toxic effect of IPN is hepatic damage with jaundice. We observed the latter in 3 cases, 2 mild and 1 fatal. This last patient had been under treatment for 3 months, and jaundice appeared 10 days after he had stopped taking the drug. Eight days after the first signs of jaundice appeared he lapsed into coma and died. The necropsy findings were as follows:

Macroscopic. Weight of the liver was 1250 gm., form was preserved, consistency was inferior to normal, external surface was slightly wrinkled, and of a reddish brown color, brilliant, and uniform; cut surface was yellowish brown, smooth, and of friable consistency; thin slices could be obtained easily. Lobulette formation was not distinguished. Bile ductus was permeable and without change.

Microscopic. Both lobules were similar in aspect. The architecture had disappeared. There was diffuse necrosis of the hepatic cells and inflammatory infiltration of a chronic type. The majority of the portal spaces present moderate infiltration of lymphocytes and proliferation of bile ductules. In some there were deposits of bile pigment that may also be seen in areas of parenchyma. The sinusoids are dilated and show prominent Kupffer cells. No hyalin globules are identified with stains for fat; steatosis was not observed (FIGURE 10).

We include this report due to the frequency with which hepatotoxicity appears in medical literature in relation to the medication, a frequency too marked to be considered as simple coincidence. This problem requires more study, which is difficult, since no satisfactory clinical or laboratory proofs of permanent hepatic damage exist. The percentage of patients who show liver involvement is relatively small, and the question remains in our minds whether it is a problem of toxicity, of sensitivity, or of sensitization.

We have also studied 2 patients with Raynaud's syndrome, both of whom presented significant improvement in their symptomatology, with reappearance of symptoms on withdrawal of the medication. The mechanism of action in these cases must be explained by modifications in the regulation of autonomic equilibrium.

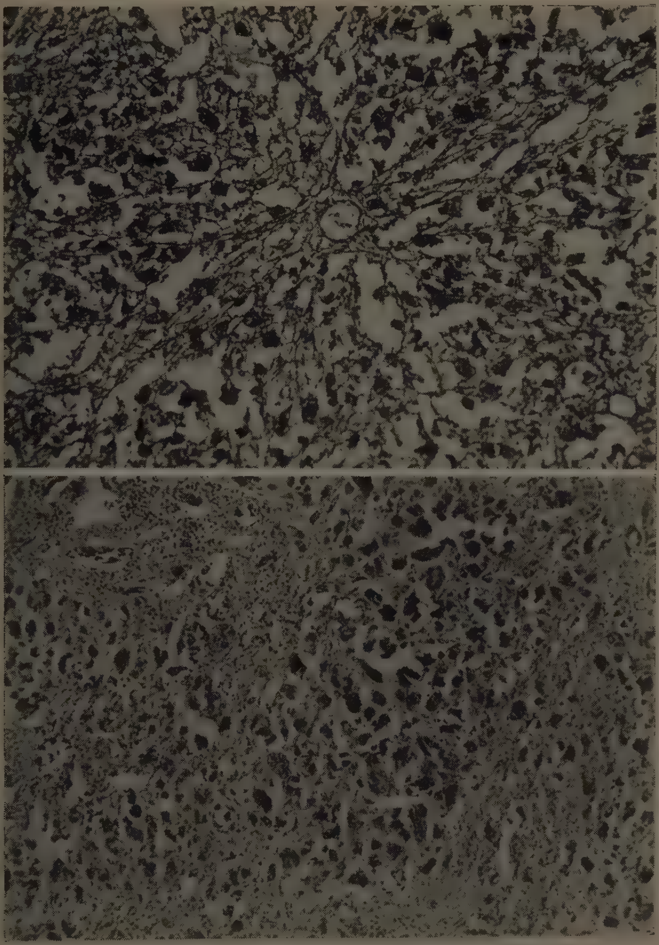

FIGURE 10. Liver necropsy.

Conclusions

IPN in the human being diminishes the intensity and the frequency of angina pain and increases the capacity for exertion.

The greater the severity of pain, the more pronounced is the therapeutic response.

There is a latent period for the appearance of the clinical effects of the medication.

The prolongation of effects after the withdrawal of the drug suggests a cumulative action.

The effect on pain is observed during the pharmacological action of the drug the symptomatology returning when this action is completed.

Myocardial infarctions that appeared during treatment were asymptomati

The electrocardiographic changes seen under treatment are not conclusive.

IPN is a hypotensive agent that produces fundamentally orthostatic hypotension.

The degree of its hypotensive effect bears a direct relationship to the magnitude of the pretreatment blood pressure.

Hypotension, independently of its degree, produces no exacerbation o anginal pain. Nevertheless, in many instances, unfavorable electrocardiographic changes related to this fall in blood pressure were observed. Thes consisted in the augmentation of ischemia, but never with the appearance o necrosis.

The actions on angina pectoris and on blood pressure appear simultaneousl with the rest of the pharmacological effects of the drug.

The degree of suppression of angina pain and of hypotension attained is i direct relationship to the intensity of side effects.

The side effects are less intense when diminution of angina pain is attaine than when the blood pressure drop becomes significant.

Through analysis of the clinical effects of IPN it may be concluded that th drug has a prolonged reversible ganglioplegic action.

The hypotensive effect of IPN is due to its ganglioplegic action.

The hypothesis that the modification of angina symptomatology is due to ganglioplegic effect is proposed.

The intensity and duration of the ganglioplegic effect of IPN could rende it particularly useful in the treatment of angina pectoris and of high bloo pressure, especially when these conditions are present simultaneously.

The simultaneity of therapeutic effect and undesirable side effects curtai the practical usefulness of the drug.

The possible hepatotoxic effects of IPN should be taken in account and mer further study.

Due to the limitation imposed by its side effects and its possible toxicit the following indications are suggested for IPN in cardiovascular therapy (1) severe angina pectoris (Grades 3 and 4) with high blood pressure; (2) seve angina pectoris (Grades 3 and 4) without high blood pressure; and (3) essentia hypertension that has not responded to other forms of treatment.

Acknowledgment

The iproniazid (IPN) used in this investigation was obtained as Marsili through the courtesy of José Guardiola from Productos Roche S. A., Mexic D. F., Mexico.

Reference

1. Cesarman, T. 1958. Marsilid in the treatment of angina pectoris. J. Clin. Expt Psychopathol. & Quart. Rev. Psychiat. Neurol. **19:** Suppl. 1: 169.

CONTRIBUTION OF IPRONIAZID TO THE TREATMENT OF ANGINA PECTORIS

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The treatment of angina pectoris is fundamentally symptomatic because of the impossibility of modifying its basic cause—coronary atherosclerosis. While the problem of pain control may be solved with fast-acting nitrites and, if necessary, with morphine or similar drugs, its prevention is a different thing altogether.

Innumerable medications and even surgical measures have been recommended for this, some of which are daring and not devoid of danger. Year after year, however, experience has proved the inefficacy of these methods and, although they enjoyed great favor at first, ultimately they have been forgotten. What remains fundamental in the therapy is partial or complete restriction of physical activity and, within human limitations, of emotional states and, according to the severity of pain, the use of nitroglycerin to control it.

To this must be added, as adjunctive routine measures, long-acting nitrites and phenobarbital, anticoagulants and, only under certain conditions, antithyroid drugs and even opiates, the latter in spite of the risk of habituation.

Results are not always satisfactory, however, and the great majority of patients must resign themselves to spending the rest of their lives with increasing physical incapacity and to the daily use of several nitroglycerin tablets.

For this reason, the announcement of the discovery of a substance that improves such a situation, even partially, leads to repercussions, not only in medical circles but also among the laity, who acquire their information from the popular press.

The findings of Cesarman[1] and Cossio[2] that iproniazid phosphate, in doses of 50 mg. every 8 hours, prevents anginal pain totally or partially in a highly significant percentage of patients opened up new possibilities for the treatment of this symptom of coronary disease.

Thus, in my first experience with 36 patients suffering from angina pectoris of different severity, administration of iproniazid in the above-mentioned dose prevented pain in 78, 75, and 50 per cent in Grades I, II, and III,* respectively. Relief of pain usually occurred between the third and the seventh days after the first administration of the drug. This occasionally occurred as early as the first day, but sometimes as late as the second week of treatment. These salutary effects persisted throughout the period of medication and even for 2 to 3 weeks after the drug was discontinued.

Such percentages of good results have been repeatedly confirmed, with one exception (Lozada et al.[3]) by several authors in different countries (Schweizer,[4] Barrera and Garcia-Turiella,[5] Galicer,[6] Dighiero *et al.*,[7] and Binda and Fasoli[8]).

However, iproniazid in the above-mentioned doses also brought on several undesirable side effects: constipation, insomnia, dysuria, nausea, weakness, frustrated orgasm and impotence, and transient hypoprothrombinemia (Cossi and Pecorini[9]). In addition, as a result of orthostatic hypotension, there is th danger of fainting and syncope with or without occlusive vascular accidents In the majority of cases these effects are controllable 2 to 15 days after cessation of treatment. The occurrence of these untoward reactions has raised question as to the practical value of iproniazid in the treatment of angina pectoris.

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* Grade I, pain produced only by "selective" effort; Grade II, pain on least effort and sometimes spontaneously at rest or even during sleep; Grade III, spontaneous and repeated paroxysmal pain.

Fortunately, this first personal experience had demonstrated that the collateral effects generally occurred some time after the pain was alleviated, in spit of decreased dosage. This indicated that there was a threshold above whic the effective dose became excessive as a consequence of the accumulation o the drug in the body. Clearly, this daily dose exceeded the daily eliminatio and metabolic degradation of the drug.

To establish the effective dose, 84 patients with angina pectoris (Cossio[10] were given decreasing or increasing doses over a period of 2 to 3 weeks, th upgrading method being more frequently used in older patients or in those wit greater heart damage, because they are more sensitive. It was thus possibl to reduce the side effects to 40 per cent and to avoid interruption of treatmen

By the time the dose was thus adjusted, various patients had taken the dru for more than six months. Late side effects were moderate anemia, easil controllable, and an atypical cardiac failure. The latter was considered du to iproniazid because myocardial infarction was ruled out; there was disproportion between the lung congestion or edema and the slight or nonexistent cardia enlargement, and gallop rhythm and functional mitral insufficiency were absent

Because our experiences in the studies mentioned above showed that patient under iproniazid treatment became more sensitive to fast-acting nitrites, requiring less nitroglycerin to control pain, and because they were sometime subject to an orthostatic syncope, it was decided to use the drug with slow acting nitrites in 180 other anginal patients of the chronic type—that is, Grade I and II (Cossio[10]).

This combination of drugs was used for another reason: to increase th coronary flow as well as to bring about relief of pain through the action of iproniazid. In this manner iproniazid, while reducing pain, does not produce th alarming symptom of ischemia.

The results have been satisfactory. Less iproniazid was needed to contro pain, undesirable side effects have been reduced to 25 per cent of the cases, an it was necessary to interrupt the treatment only rarely. Figures 1, 2, and illustrate the effect of iproniazid in the course of treatment of 3 typical case with histories of angina of 2 to 16 years' duration.

The iproniazid treatment of angina pectoris devised in this investigatio follows this regimen:

(1) Physical activity is restricted and agitation avoided, depending on th condition of each patient.

(2) Tobacco is excluded.

(3) The diet is adapted to the needs of the individual, with restriction o ingestion of fats and strong alcoholic beverages.

(4) Nitroglycerin is used to control pain (with a warning to the patient no to take it while standing), and morphine is used only when necessary.

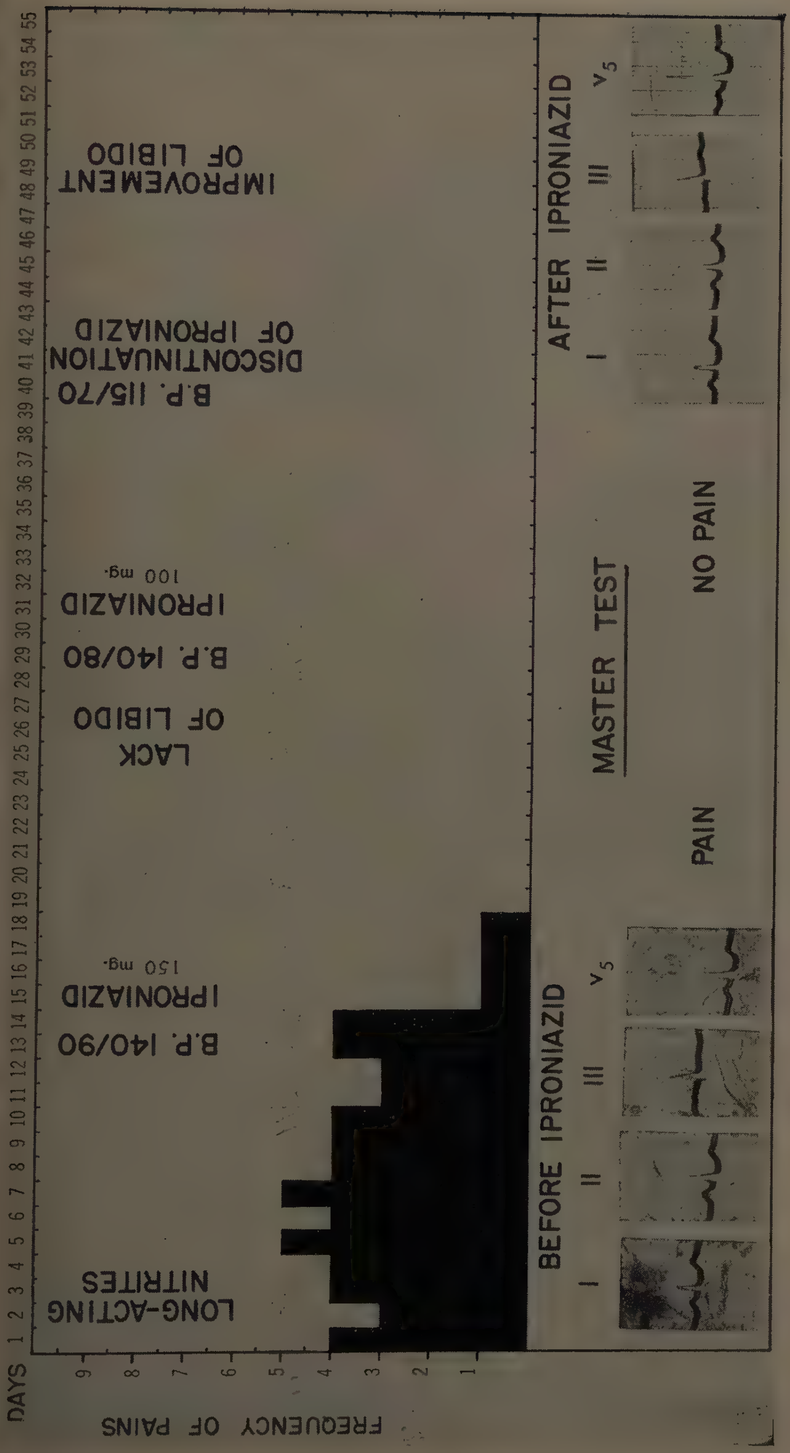

Figure 1. Effect of iproniazid. Patient J. P., 47 years old, with angina pectoris.

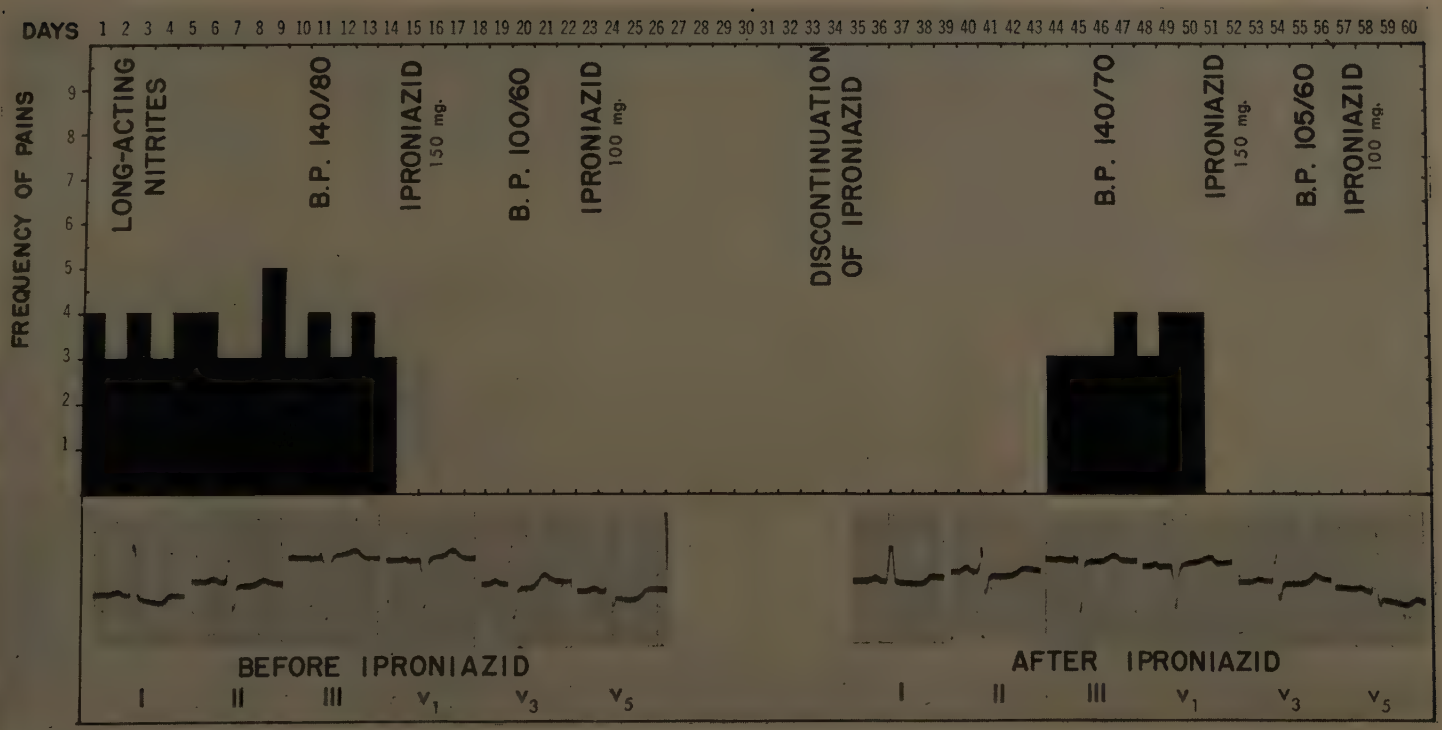

FIGURE 2. Effect of iproniazid. Patient H. de L., 68 years old, with angina pectoris of 8 years' duration and a myocardial infarct suffered in August 1949.

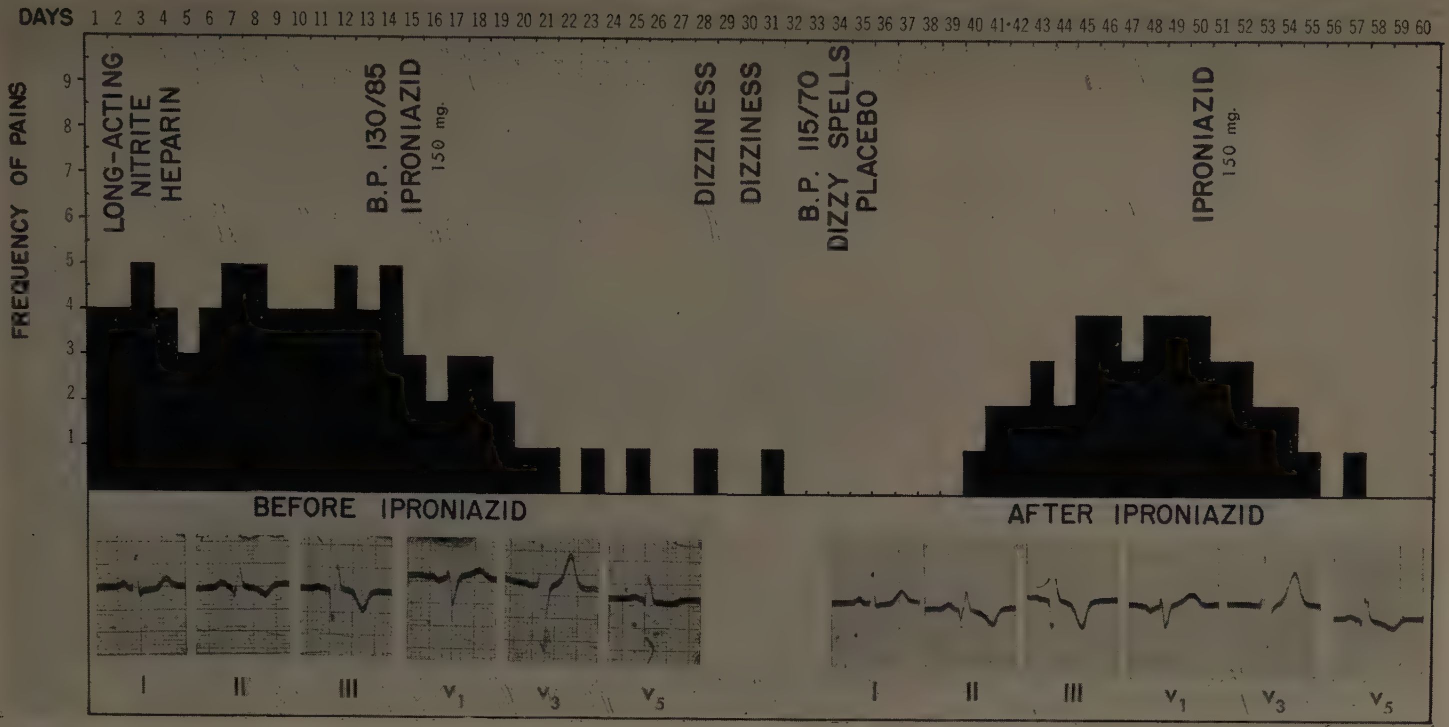

FIGURE 3. Effect of iproniazid. Patient G. M., 52 years old, with angina pectoris of 16 years' duration and 2 myocardial infarcts, 1 suffered in 1940, the other in 1957.

(5) Anxiety, arterial hypertension, anoxia, and similar states, are avoided and if possible, are treated.

(6) Nitrites of prolonged action are administered every 4 hours, generally with phenobarbital.

(7) Simultaneously (or after 2 weeks, according to the requirements of the patient and the possibilities of supervision) 50 mg. of iproniazid is administered every 8 hours to those patients under 60 years of age, and with little heart damage, and every 12 hours to those older than 60 who exhibit physical signs of cardiac damage. These doses are given for only 2 or 3 days, with subsequent reduction to 50 mg. every 12 and 24 hours, respectively, until the very first relief from pain appears. Then the dose is reduced further to 50 and even 25 mg. daily, according to results obtained. Iproniazid is continued indefinitely, as long as the occurrence of undesirable side effects does not necessitate immediate discontinuance of medication either temporarily or permanently.

(8) Administration of iproniazid is interrupted after 2 or 3 months of constant used, as the relief of pain may persist without medication. Only when pain recurs should medication be reinstituted.

In impending infarction, the use of iproniazid is justified only after 2 or 4 weeks of persistent paroxysmal pain despite such measures as complete bed rest and anticoagulants. However, if iproniazid is used, arterial pressure must be watched closely, not only in the supine position, but also in the standing position, since a marked drop in pressure increases myocardial ischemia with exacerbation of pain and the danger of occlusive coronary as well as cerebral vascular accidents.

Summary

The treatment with iproniazid phosphate of 300 patients with angina pectoris since August, 1957, has shown the following:

In 2 of 3 cases, 50 mg. of iproniazid every 8 hours prevents pain, partially or totally, within 4 to 10 days. Undesirable effects occur in 60 per cent of the cases.

The alleviation of pain generally continues after the dose is decreased to 50 mg. every 12 or 24 hours; at this dose level the undesirable effects diminish or disappear.

While administering iproniazid, pain from usual causes does not appear or if it does appear, can be controlled with smaller doses of nitroglycerin. If the cause of the pain is aggravated, pain may reappear with the same, or even greater, intensity.

The older the patient and the greater the heart damage, the more frequent and intense are the undesirable effects; the complication of cardiac insufficiency may arise, but is easily controlled even without discontinuance of iproniazid.

With doses of 50 mg. daily, side effects are unusual, even if medication is continued for several months.

With smaller doses of iproniazid, pain decreases and side effects are eliminated if long-acting nitrites are administered adjunctively.

Iproniazid has the following characteristics: (1) it is readily absorbed; (2) its effects are cumulative and persistent; (3) the dose sufficient to control pain is slightly less than the dose that produces undesirable effects, and the latter

may be controlled by discontinuance of the drug; (4) iproniazid can be potentiated by nitrites; (5) it is capable of prolonged effect; (6) it does not modify the evolution of the coronary disease; and (7) it does not produce changes in the electrocardiogram, except those changes related to the hypotensive effect of iproniazid.

A satisfactory regimen involves the following: exclusion of tobacco; physical and dietary restrictions that must be maintained always, regardless of the absence of pain; long-acting nitrites; anticoagulant medication in acute or subacute cases; iproniazid at dosages as outlined above; and, finally, nitroglycerin if necessary.

Acknowledgment

The iproniazid phosphate (Marsilid) used in our investigations was supplied by Productos Roche S.A., Buenos Aires, Argentina.

References

1. Cesarman, T. 1957. Serendipitia y angina de pecho. Informe preliminar sobre un hallazgo terapéutico. Arch. inst. cardiol. Méx. **27:** 563.
2. Cossio, P. 1957. Tratamiento de la angina de pecho y otros dolores musculares isquémicos por el fosfato de iproniazida. Prensa méd. arg. **44:** 2679.
3. Lozada, B. B., N. Tempone, J. Muniagurria & D. J. Vicario. 1958. La iproniazida en la angina de pecho y en la claudicación intermitente. Semana méd. **113**(4): 157.
4. Schweizer, W. 1958. Über die Wirkung von Isopropyl-isonicotinsäurehydrazid bei Angina pectoris. Cardiologia. **33:** 40.
5. Barrera, J. A. & R. Garcia-Turiella. 1958. Tratamiento del síndrome anginoso. Resultados del empleo de la iproniazida. Prensa méd. arg. **45:** 1560.
6. Galicer, R. F. Consideraciones sobre el tratamiento de la angina de pecho con el fosfato de iproniazida. Prensa méd. arg. In press.
7. Dighiero, J., J. Hazen, O. V. Aguirre & J. Rudolf. 1958. La iproniazida (Marsilid) en el tratamiento del dolor anginoso. El Dia Méd. Uruguayo. No. **302:** 2192.
8. Binda, G. & A. Fasoli. 1958. Sull'azione della isonicotinilisopropilidrazide nell'angina di petto. (Nota preliminare). Folia Cardiol., **17**(2): 119.
9. Cossio, P. & V. Pecorini. 1958. Iproniazida y concentración de protrombina. Prensa méd. arg. **45:** 1776.
10. Cossio, P. 1958. The treatment of angina pectoris and other muscular pain due to ischemia with iproniazid and isoniazid. Am. Heart J. **56:** 113.
11. Cossio, P. 1958. Traitement de l'angine de poitrine avec l'iproniazide et l'isoniazide. III^e Congrés Mondial de Cardiologie. Brussels, Belgium.

ISOPROPYLHYDRAZIDES IN ANGINA PECTORIS

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In September 1957, stimulated by the observation of Cesarman[1] and Cossio,[2] my colleagues and I began to investigate the effect of isopropylhydrazides in angina pectoris. So far we have had experience with four different isopropylhydrazides, and some of our findings[3, 4] have been published.

Iproniazid (Marsilid*) was given for 12 to 342 days to 100 ambulatory patients with angina pectoris. The initial dosage was 150 mg./day. The pain on effort disappeared entirely in 48 per cent of the patients and, in 49 per cent, a variable decrease in frequency and intensity of pain was noted; the pain on effort remained unchanged in 3 per cent. The corresponding figures for pain at rest were 63, 28, and 9 per cent. This effect did not seem to be influenced by the duration or the severity of the disease or by the change in arterial pressure. The alleviation of pain began after 2 to 18 (average 7) days. The maximal effect was reached, in the majority of cases, before the twentieth day and remained constant during the trial period. An increase of the daily dosage to 200 mg. in 12 patients was not followed by any further improvement. The dosage was lowered to 100 mg./day in 30 patients, and after a few days, 20 of these complained of an increase in pain. The electrocardiogram at rest, normal in 26 cases at the beginning of the trial, was followed regularly in 7 patients; no changes were observed. The electrocardiogram was pathological in 74 patients and was repeated in 63; in 33 it remained unchanged. In 21 patients the changes suggestive of strain or ischemia became less pronounced whereas, in 9, the reverse occurred. There seemed to be no relationship between the effect on pain and changes in the electrocardiogram; some examples are shown in FIGURE 1.

The arterial pressure dropped during the trial period in 11 of 44 normotensive and in 41 of 56 hypertensive patients. A decline of 20 mm. Hg in the systolic and diastolic pressure was considered significant; differences of as much as 60 mm. Hg were observed. Orthostatic hypotension was frequent, and the change in arterial pressure was noted at the same time as the change in pain. In 19 patients there seemed to be a decrease in hypotension after 50 to 70 days. Serious complications and undesirable side effects were observed during the test. The complications were: myocardial infarction (4 cases), congestive heart failure (cases), cerebral vascular accident (2 cases), urinary retention (2 cases), psychosis (2 cases). The myocardial infarctions occurred 13, 15, 32, and 90 days after beginning treatment. In each of these cases iproniazid had abolished the antecedent anginal pain entirely. Symptoms of the infarct (pain, electrocardiographic alterations) were typical; two of the patients died. Congestive heart failure was observed in 4 patients after treatment periods of 20, 37, and (in 2 patients) 90 days. These patients had had considerable cardiac enlargement before treatment, but no evidence of failure. The cardiac failure remained unexplained, especially since uncontrolled tachycardia and myocardial infarction did not occur and there were no output-elevating factors. Signs of cerebral arteriosclerosis had been noted before the beginning of the trial in the 2 patients who suffered a cerebral vascular accident. In 1 of these, considerable hypotension was observed immediately after the onset of the attack, and 1 patient died. Side effects were noted in 89 per cent of the cases. The most frequent complaints were sleeplessness, impotence, constipation, vertigo, and fatigue. In 3 patients with paroxysmal auricular fibrillation and an elevated metabolic rate, the paroxysms became less frequent and the metabolic rate was lowered 20 per cent or more.

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* Hoffmann-La Roche.

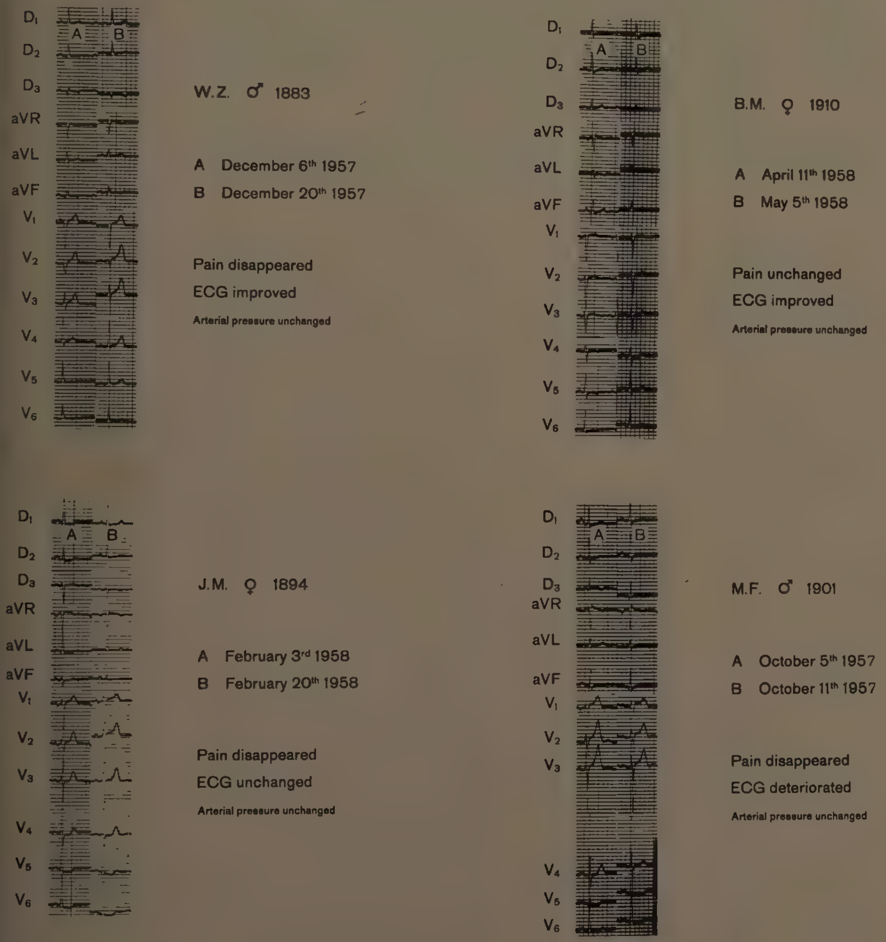


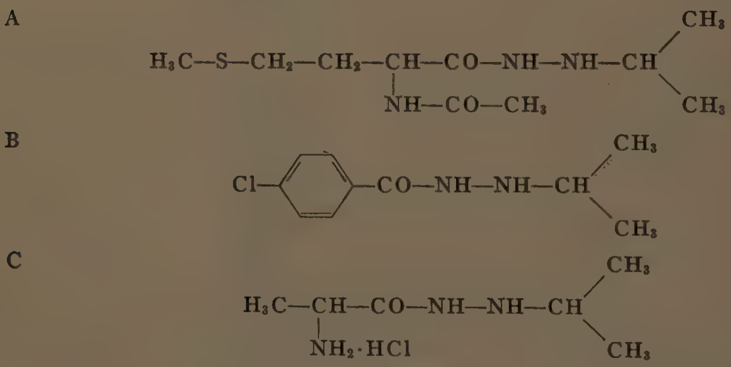
FIGURE 1

The mechanism of action of iproniazid is still unknown. It is improbable, however, that the effect on anginal pain is caused by a diminution of the myocardial ischemia. There is no relationship between the change in pain and the changes of the electrocardiogram,[2-6] and little is known about the spontaneous variation in the electrocardiogram in coronary heart disease. Published investigations on the effect of iproniazid on the electrocardiogram after effort or during hypoxia are few, and the results are contradictory.[1, 5-8] Insofar as I know, the effect of iproniazid on the ballistocardiogram after smoking has not been published, and the significance of the observed lowering of the metabolic rate remains to be explained. It is improbable that iproniazid has any beneficial effect on the natural course of coronary heart disease. The incidence of myocardial infarction during the administration of iproniazid has not been surprisingly low or unexpectedly high.[1-8] Iproniazid does not modify symptoms or signs of myocardial infarction.

TABLE 1
REASONS FOR DISCONTINUING IPRONIAZID*

Cause of interruption	Number of cases
Serious complications	13
Unwanted side effects	50
Inefficiency	3
Disappearance of pain	12
Lack of collaboration	16

* Six patients continued with iproniazid.



A: $H_3C-S-CH_2-CH_2-CH(NH-CO-CH_3)-CO-NH-NH-CH(CH_3)_2$

C: $H_3C-CH(NH_2\cdot HCl)-CO-NH-NH-CH(CH_3)_2$

FIGURE 2

Although iproniazid may have only a symptomatic effect, it is still a very useful drug for patients suffering from angina pectoris without cerebral arteriosclerosis or significant cardiac enlargement, as long as side effects are tolerated and excellent cooperation exists between patient and doctor. Unfortunately, these conditions are fulfilled in only a small number of cases. At the time of this report, only 6 per cent of our patients were still taking iproniazid. The reasons for ending the trial are shown in TABLE 1. Even if it is possible, by lowering the initial dosage, to increase the number of cases that can be treated satisfactorily, the development of better tolerated drugs is necessary.

Thus far, each of the following 3 new substances* has been tested for at least

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* Received from F. Hoffmann-La Roche & Co., Ltd., Basel, Switzerland.

20 days in 10 to 15 patients previously treated with iproniazid. DL-acetyl-methionyl-N^2-isopropylhydrazine and N^1-(p-chlorobenzoyl)-N^2-isopropylhydrazine (FIGURE 2) are amine oxidase inhibitors that, like iproniazid, increase the catecholamine content of heart muscle, but do not influence the arterial pressure in the cat. In patients with angina pectoris the effects of both substances have been shown to be almost identical to those of iproniazid. The third compound, L-α-alanyl-isopropylhydrazine (FIGURE 2), is a stronger amine oxidase inhibitor than iproniazid. Unlike iproniazid, it increases arterial pressure in the cat and has a positive inotropic effect. In patients with angina pectoris the effective dose of this substance was found to be smaller than that of iproniazid (50 to 75 mg. per day). Some of the side effects were less severe, but unfortunately the hypotensive reaction was pronounced.

References

1. CESARMAN, T. 1957. Serendipitia y angina de pecho. Informe preliminar sobre un hallazgo terapeutico. Arch. inst. cardiol. Méx. **27:** 563.
2. COSSIO, P. 1957. Tratamientode la angina de pecho y otros dolores isquemicos por el fosfato de iproniazida. Prensa méd. arg. **44:** 2679.
3. SCHWEIZER, W. 1958. Über die Wirkung von Isopropyl-isonikotinsäurehydrazid bei Angina pectoris. (Vorläufige Mitteilung.) Cardiologia. (Basel). **33:** 40.
4. SCHWEIZER, W. & P. v. PLANTA. 1958. Über die Wirkung von Isopropyl-isonikotinsäurehydrazid bei 100 Fällen von Angina pectoris. Schweiz. med. Wochschr. **88:** 882.
5. COSSIO, P. The treatment of angina pectoris and other muscular pain due to ischemia with iproniazid and isoniazid. Am. Heart J. **56:** 113.
6. MASTER, A. M. 1958. Iproniazid (Marsilid) in angina pectoris. Am. Heart J. **56:** 570.
7. CESARMAN, T. 1958. Marsilid in the treatment of angina pectoris. J. Clin. Exptl. Psychopathol. Spec. Suppl. **19:** 169.
8. BINDA, G. & A. FASOLI. 1958. Sull'azione della isonicotinilisopropilidrazide nell'angina dí petto. (Nota preliminari). Folia Cardiol. **17:** 119.

IPRONIAZID IN THE ANGINAL SYNDROME DUE TO CORONARY DISEASE

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Since Cesarman's[1, 2] serendipitous discovery that iproniazid (Marsilid*) dramatically relieved anginal pains in cardiac patients treated for their depression, Cossio[3] in Buenos Aires, and more recently, Schweizer and Planta[4] in Basel have reported clinical trials designed to study the direct effect of iproniazid on angina pectoris. All three investigators reported complete relief of pain in considerably more than one half of the patients and some relief in most of the rest. The number and intensity of anginal attacks were reduced often within several days even in patients who previously required high doses of nitroglycerin daily. The capacity for exertion increased, while such side effects as were noted were mild and could be controlled easily by reduction in dosage of the drug. The point of difference between the results of Cesarman,[1, 2] of Cossio,[3] and of Schweizer and Planta[4] was in the effect on the electrocardiogram. Cossio[3] failed to note any improvement either in abnormal resting electrocardiogram or in the results of the Master[5] "2-step" test, and Schweizer and Planta[4] found, in addition, that the ischemic changes decreased in 30 per cent and increased in 13 per cent of the cases. The arterial pressure fell, both in normotensive and hypertensive patients, and 40 per cent of the patients in the Swiss study showed orthostatic hypotension.

In all 3 studies, the dose of iproniazid was 50 mg. t.i.d., after meals, for periods ranging from several days to 7 months. In the majority of cases the effective dose was 150 mg./day. While Cesarman[1, 2] suggested that iproniazid probably acts directly on the etiological factors producing angina pectoris, the others are of the opinion that the myocardial ischemia or the natural course of the disease is unaltered by iproniazid.

In view of these promising results in the treatment of angina pectoris, which incapacitates millions of people, we deemed it desirable to subject iproniazid to further clinical tests, and to attempt to resolve the difference in points of view taken by Cesarman[1, 2] and Cossio[3] as to the mechanism of action.

Clinical Material and Methods

The present study was made on 74 patients suffering with typical severe angina pectoris. The age range was 50 to 70 among 58 men and 16 women (TABLE 1). Six of the patients were in "status anginosus" and required 20 to 60 tablets of nitroglycerin daily. Most of the remaining patients had 2 to 20 attacks daily, and 6 who were able to avoid precipitating factors experienced only a few episodes each week.

Each patient was given 50 mg. of iproniazid 3 times daily after meals. In addition, patients received 25 mg. pyridoxine with each dose of iproniazid, with a view to preventing the development of a peripheral neuritis. Vasodilators, with the exception of nitroglycerin, were not used. In cases of heart failure the usual measures of treatment were employed.

* Hoffmann-La Roche.

The course of treatment with iproniazid was carried out from 1 week to 5 months. Of the 74 patients under clinical trial, 5 have been on the drug for almost 11 months. Severe side reactions compelled the discontinuance of iproniazid in the remaining patients while, in some, the drug was tolerated for 1 month or less.

TABLE 1
AGE AND SEX DISTRIBUTION OF 74 PATIENTS TREATED FOR ANGINA PECTORIS WITH IPRONIAZID*

Age in years	No. Males
40–49	8
50–59	26
60–69	18
70–79	6
	58
	Females
40–49	1
50–59	2
60–69	9
70–79	4
	16

* Average age of males: 59 years; of females: 63 years.

TABLE 2
RELIEF OF PAIN (74 PATIENTS) BY IPRONIAZID IN ANGINA PECTORIS

Degree	No. of patients
Complete	13
Almost complete	28
Doubtful	3
Relief, then aggravation	1
No relief	29
Total	74

Results

Thirteen patients of the 74 were completely relieved of angina and were able to perform normal tasks with confidence and without any pain. In 28, cardiac pain was almost completely eradicated, except for occasional recurrence with severe exertion (TABLE 2). In 3 cases improvement was doubtful and, in 29, the chest pain was not relieved. In 1 patient improvement was followed by an aggravation of the symptoms. Summarizing these results, complete or almost complete relief of the anginal syndrome occurred in 41 patients, representing more than one half of our patients.

When iproniazid was effective, symptoms were relieved within 3 to 10 days, and improvement was sustained as long as medication was continued. When this was stopped, the antianginal effect of the drug usually persisted for periods ranging from a few days to 2 weeks; it then disappeared slowly. In some instances, the beneficial effects were maintained for weeks and, in several cases, for 8 months to date, despite discontinuance of the drug.

Electrocardiograms before and after treatment were available in 28 cases. In this group, 22 had experienced dramatic improvement or relief of symptoms, 5 showed no symptomatic change, and one patient deteriorated clinically after an initial favorable response. Only 2 patients in the group showed any improvement in the abnormal resting electrocardiogram, while 4 with an abnormal electrocardiogram prior to treatment presented a slight or equivocal electrocardiographic improvement. However, in all of these there was evidence of developing improvement even prior to iproniazid therapy, which complicates the interpretation. In 20 of the 28 patients, the abnormal resting electrocardiogram remained completely unchanged after treatment (TABLE 4 and FIGURE 1). In 2, there was definite deterioration of the resting electrocardiogram and, in 2 of these, an acute coronary occlusion occurred.

In the 6 patients whose resting electrocardiogram was normal, the Master[5] 2-step test was positive. Although there was clinical improvement again, there was no change in the record after iproniazid treatment (TABLE 4 and FIGURE 2).

Side Effects

Fourteen of the patients showed no side effects. Untoward reactions categorized according to the system most likely to have been involved are listed in TABLE 3. The gastrointestinal effects were constipation, "indigestion," flatulence, distention, abdominal discomfort, belching and passing of wind, and intractable dryness of mouth. The last of these effects and constipation occurred in about one third of the patients.

The genitourinary disturbances included difficulty in micturition, polyuria, dysuria, nocturia (19 subjects), and impotence (7). Of the 7 with impotence, 2 elected to continue the drug and 2 discontinued treatment; the remaining 3 refused the drug because of other side effects. In those who discontinued taking the drug the impotence disappeared in 1 or 2 days.

Dizziness, lightheadedness, and slight faintness occurred in 25 patients. Grouped as cardiovascular reactions together with distinct blood pressure drop in 4 patients, and syncope in 3 others, this side reaction was perhaps the most common and most important. In the 4 whose blood pressure fell without producing syncope, the readings were 80 to 94 mm. Hg systolic, and 50 to 60 mm. Hg diastolic.

Nine patients showed definite psychoenergizing effects; insomnia occurred in 5 and headache in 6. However, 6 patients were profoundly fatigued, and 3 of these became very sleepy; such symptoms had not previously been experienced by these 6 patients.

Four patients developed severe peripheral neuritis. In 1 with a 3-year history of neuritis in the right lower extremity following a hemiplegia, the pain was much intensified by iproniazid, but the angina pectoris had disappeared and he has remained on the drug for 5 months. Severe muscle twitching and "shakes" developed occasionally.

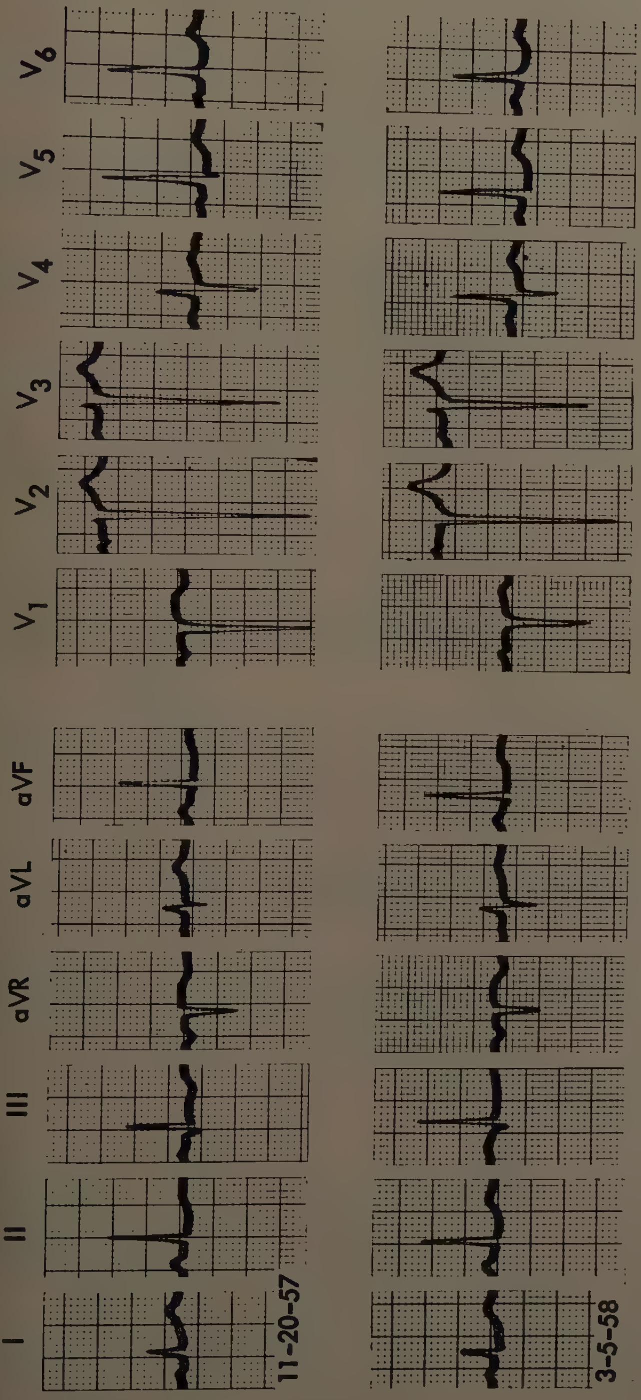
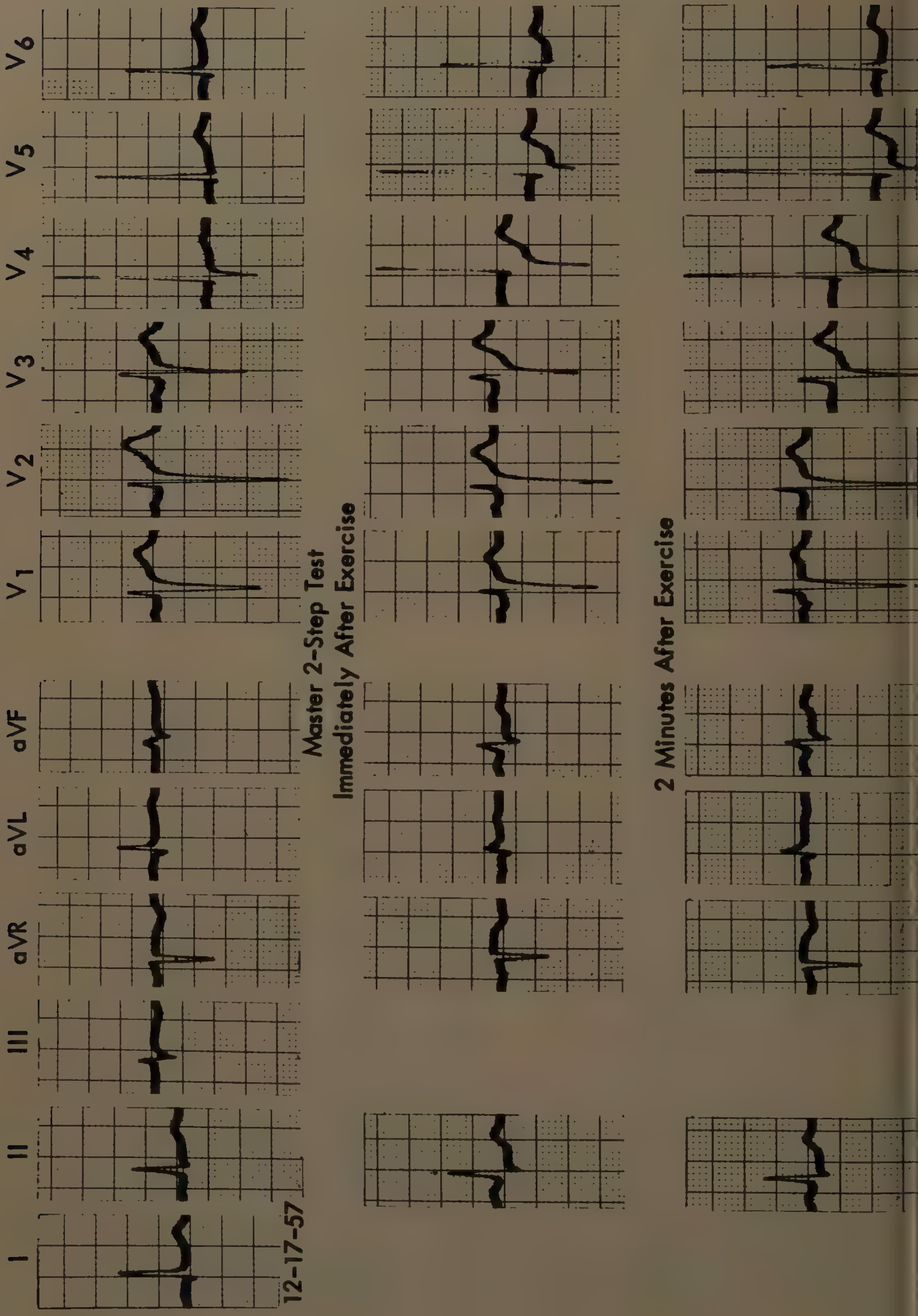


FIGURE 1. Resting electrocardiogram for patient S.I., male, 48. Status anginosus; patient had suffered coronary occlusion once and coronary insufficiency twice. *Q* in V_1, *rS* pattern in $V_{1,2}$ with *RS-T* segment depressions in II, III, aVF, and $V_{5,6}$. No change after 5 months of iproniazid.


I
II
III
aVR
aVL
aVF
V1
V2
V3
V4
V5
V6
12-17-57
Master 2-Step Test
Immediately After Exercise
2 Minutes After Exercise

Profuse sweating occurred in 3 patients, but did not necessitate discontinuance of the drug. Appetite was increased and 4 patients gained weight, 1 overweight patient gaining 20 lb. in 4 months of treatment. One case of hepatitis occurred, from which the patient recovered.

TABLE 3
SIDE EFFECTS OF IPRONIAZID IN TREATMENT OF ANGINA PECTORIS

Effect	No. of patients
Gastrointestinal	
Constipation	25
Flatulence	8
Dryness of mouth	21
Hepatitis	1
Genitourinary	
Urinary disturbances	19
Impotence	7
Cardiovascular (?)	
Dizziness	25
Blood pressure drop	4
Syncope	3
Cerebral (?)	
Cerebral stimulation	9
Headaches	6
Insomnia	5
Fatigue and drowsiness	6
Neurological	
Neuritis	4
Miscellaneous	
Weight gain	4
Diaphoresis	3
No side effects	14

TABLE 4
EFFECT ON ELECTROCARDIOGRAM AND MASTER 2-STEP TEST OF IPRONIAZID IN ANGINA PECTORIS

Control electrocardiogram	
No improvement	20
Improvement	6
4 Slightly improved, but had been improving prior to treatment	
2 Definitely improved, but had been improving rapidly prior to treatment	
Aggravation	2
1 Coronary occlusion	
1 Definitely worse	
Master 2-step	6
Unchanged 6	

Discussion

Without doubt, the complete or nearly complete relief of chest pain in 41 of a series of 74 seriously sick patients (including complete relief in 5 of 6 with

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FIGURE 2. Master 2-step test result for patient R.A., male, 45. The patient suffered with angina pectoris for many years. The resting electrocardiogram had been normal, but the 2-step was always abnormal. The test was as significantly positive after 4 months of iproniazid therapy as before.

"status anginosus") is remarkable testimony to the effectiveness of iproniazid in alleviating subjective symptoms of angina pectoris. Our experience confirms the observation of previous investigators that it is the most promising drug yet available for this indication. However, the toxic side effects accompanying its use regrettably limit it at the present time to those patients in whom desperate measures are justified.

One can only speculate on the mechanism of action of iproniazid in its effect on anginal pain. Its known action in inhibiting monoamine oxidase, an enzyme involved in the breakdown of serotonin, epinephrine, norepinephrine and, possibly, other amines[6] may account for its stimulating effect on the central nervous system; an effect that would be exercised through its sparing action on these amines in the brain. Iproniazid apparently prevents the serotonin in the brain and, possibly, in the heart from being destroyed by monoamine oxidase. Just how it produced symptomatic relief of angina pectoris is not known. While serotonin does have certain cardiovascular effects, it is doubtful that it relieves the pain in angina pectoris.[6, 7, 8] Reserpine, which also increases the supply of serotonin in the body, does not relieve angina pectoris. There remains the possibility that the relief of chest pain is brought about through the autonomic nerve connections between the heart on the one hand and the brain and spinal cord on the other.

Pletscher and Pellmont[9] have demonstrated a striking rise in the catecholamine content of the heart of guinea pigs treated with iproniazid, an effect that could be related to the observed therapeutic response. At the same time Raab[10] advances the theory that the catecholamines, that is, epinephrine and norepinephrine, are the factors that produce rather than relieve pain in cardiac disease.

Cesarman[1, 2] proposes that iproniazid may influence cardiac metabolism through some enzymatic mechanism and, possibly, thus relieve pain. Cossio[3] postulates that the antianginal attack is due to a selective block of the "P" substance of Lewis, the pain-producing agent liberated under conditions of ischemia. Schweizer and Planta[4] do not think that iproniazid has any effect on the myocardial ischemia or on the disease process.

Our experience causes us to favor the idea that iproniazid relieves anginal pain at least partly through cerebral stimulation, by elevating mood and increasing the pain threshold. In our study the greatest benefit seemed to occur in the most apprehensive patients, in whom the "psychic energizer" effect may have been dominant.

Many of the side effects of iproniazid seem to resemble those of antihypertensive ganglion-blocking agents, yet these drugs do not relieve anginal pain. The gastrointestinal and genitourinary manifestations may be attributed to alterations in autonomic nerve balance. Mental exhilaration, insomnia, reduced requirements for sleep,[11] hyperreflexia, muscular twitching and tremors, and peripheral neuritis[12-14] reflect the effects of iproniazid on the central and peripheral nervous system. The simultaneous administration of 25 mg. of pyridoxine minimizes and, in some instances, may actually prevent peripheral neuritis, dryness of the mouth, and disturbances in urination.

Dizziness may be the result of direct brain stimulation or interference with labyrinthian function. A pronounced orthostatic effect has been observed in hypertensive patients.[15] A blood-pressure decrease may well have been the cause of syncope with death in one of Cossio's[3] patients and a factor in the sudden death from coronary occlusion in another. We have shown that a fall in blood pressure during shock may precipitate coronary occlusion[16] through alteration in the coronary circulation and consequent thrombosis. This mechanism[17] may also account for coronary occlusion in two of our patients. Iproniazid may cause the drop in blood pressure through its effect on the blood-pressure center in the medulla. However, three cases of coronary insufficiency and two of coronary occlusion in seventy-four patients, nearly all of whom were gravely sick people, may well be only coincidental. While iproniazid, therefore, may not be the cause of coronary accidents, these occurrences indicate that extreme caution must be exercised in its administration to cardiac patients, since induced or spontaneous coronary insufficiency is an ever-present danger in such patients.

Since postural hypotension is not uncommon during treatment with iproniazid, the blood pressure should be taken frequently, perhaps daily, especially in those whose blood pressure is at normal levels. It should be recorded in the standing, as well as sitting or recumbent, position. In patients with regularly low blood pressure (less than 100 mm. Hg, systolic), often seen in patients who have recovered from a coronary occlusion, the drug is probably contraindicated. Medication may be resumed in smaller doses when the blood pressure has returned to safe levels. This is necessary to avoid possible cardiovascular complications, as well as the physical danger of a fall during syncope, should it occur. Normal arterial pressure levels may not be attained for 2 or 3 weeks after discontinuance of medication, since iproniazid accumulates in the body.

The patient should be cautioned to take his nitroglycerin while sitting or reclining, because the lowering of the blood pressure by this vasodilator, in addition to the iproniazid-induced hypotension, may precipitate complications. The euphoria induced by the iproniazid and the relief of angina may induce coronary insufficiency.[17] The mental exhilaration, reduced fatigue, appearance of restlessness, muscle twitching and tremors, and increased physical activity, may make additional demands on the work of the heart not commensurate with its blood supply. This is doubly dangerous if the threshold of pain is raised—whether in the brain, heart, or both.

Weight gain may be due to a "ravenous" appetite, as it was in 2 of our cases, or, it may indicate heart failure. Peripheral edema has also been reported,[18] but in our patients this may have been prevented in those with heart failure by means of dietary salt restriction and the use of diuretics.

Hepatitis, which has been associated with iproniazid therapy, occurred in only one of our patients and was followed by recovery.

The subjective improvement seen in anginal patients was remarkable, but there was no evidence of any effect on the basic coronary disease. Results with the resting electrocardiogram and the Master 2-step test[5] are important in the analysis of the mode of action of iproniazid. In none of the 28 patients for whom resting electrocardiograms were available was there unequivocal improvement after months of iproniazid therapy; 6 who showed such objective improvement had already given indications of it prior to the administration of the drug. In the 6 patients who performed the Master 2-step test[5] and were completely relieved of their anginal symptoms, there was no electrocardiographic improvement *after* the standard exercise (TABLE 4 and FIGURE 2).

Because neither the resting nor the Master 2-step electrocardiogram is altered after iproniazid therapy, the drug probably does not affect the fundamental etiological factors of coronary artery disease.

What, then, are the prospects for the usefulness of iproniazid in coronary disease? It would seem highly desirable to continue the search for an analogue of iproniazid or, perhaps, a combination with this drug, which would result in a preparation that retains the pain-relieving properties of iproniazid without its serious side effects. A drug such as ephedrine, perhaps, may be administered with iproniazid to maintain a normal blood-pressure level. Finally, a preparation that would improve the coronary circulation, increase the efficiency of oxygen utilization by the myocardium, or repair the arteriosclerosis is a desideratum meriting further research. I believe there is enough promise in iproniazid to justify intensive investigation, especially for patients in "status anginosus," where the full dosage should be employed while maintaining the precautions discussed above.

The optimum dosage has not yet been established. Most clinical investigators concur in the use of 150 mg. daily at the beginning of treatment. Certainly for a patient with "status anginosus" the full dosage should undoubtedly be used. In psychiatric cases, the manufacturers caution against the use of more than 50 mg. daily, except in severely depressed or regressed institutionalized patients. At the first sign of icterus or other serious side effect, the drug should be discontinued and, perhaps, resumed at a lower level later. It may be desirable to discontinue it in any case at intervals of 2 or 3 weeks, even in the absence of untoward reactions.

Many patients can be maintained on small doses, ranging from 10 to 50 mg. a day. Whether good results are obtainable with this dose initially has not been ascertained.

Summary and Conclusions

Seventy-four patients with severe angina pectoris were treated with iproniazid for from 1 week to 5 months; the starting dose was 150 mg. daily.

In my long experience with innumerable drugs for coronary disease not one has approached the subjective relief attained by iproniazid.

In forty-one patients, more than one half of those under treatment, iproniazid relieved the anginal syndrome completely or almost completely. Five of these patients were in "status anginosus." In twenty-nine patients, one of whom was in "status anginosus," there was no relief of pain.

Neither the resting electrocardiogram nor the Master 2-step test showed any significant improvement during the course of treatment with iproniazid, indicating that the fundamental coronary disease was not altered during the period of study.

The precise mechanism of the relief of pain by iproniazid is unknown. It may be explained by cerebral stimulation, with uplift of mood and increase of the pain threshold. Another possibility is a direct effect on the autonomic nerve connections between the heart and the spinal cord and brain.

The side effects were mainly gastrointestinal, genitourinary, and cardiovascular, the latter including dizziness, fall in arterial pressure, and syncope. Less common complications were evidences of cerebral stimulation, peripheral neuritis, dryness of the mouth, hepatitis, and weight gain. Hepatitis occurred in one patient, who subsequently recovered; four patients noted significant weight gain.

The fall in blood pressure is probably a factor in syncope and in episodes of acute coronary insufficiency, and may possibly be a factor in the precipitation of coronary occlusion.

The physical overactivity associated with euphoria, excitement, and loss of pain may cause coronary insufficiency.

The patient receiving iproniazid requires close supervision. Blood pressure should be measured in the standing position, while nitroglycerin should always be taken in the sitting or recumbent position.

In view of some of the dangerous side effects, iproniazid should be studied further by research teams in hospitals. It is hoped that investigators will search diligently for an analogue of iproniazid that will both relieve angina and be free of serious side effects.

Acknowledgment

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References

1. Cesarman, T. 1957. Serendipitia y angina de pecho. Informe preliminar sobre un hallazgo terapeutico. Arch. inst. cardiol. Méx. **27:** 563.
2. Cesarman, T. 1958. Marsilid in the treatment of angina pectoris. J. Clin. Exptl. Psychopathol. **19:** Suppl. 1, 169–177.
3. Cossio, P. 1957. Tratamiento de la angina de pecho y de otros dolores isquemicos por el fosfato de iproniazida. Prensa méd. Arg. **44:** 2679.
4. Schweizer, W. & P. v. Planta. 1958. Über die Wirkung von Isopropyl-isonikotinsäurehydrazid bei 100 Fallen von Angina pectoris. Schweiz. med. Wochschr. **88:** 882.
5. Master, A. M., L. E. Field & E. Donoso. 1957. Coronary artery disease and the "two-step exercise test". New York J. Med. **57:** 1051.
6. Zeller, E. A., J. Barsky, J. R. Fouts, W. F. Kirchheimer & L. S. Van Orden. 1952. Influence of isonicotinic acid hydrazide (INH) and 1-isonicotinyl-2-isopropylhydrazide (IIH) on bacterial and mammalian enzyme. Experientia. **8:** 349.
7. Page, L. H. & J. W. McCubbin. 1953. The variable arterial response to serotonin in laboratory animals and man. Circulation Research. **1:** 354.
8. Reid, G. 1952. Circulatory effects of S-hydroxytryptamine. J. Physiol. **118:** 435.
9. Pletscher, A. & B. Pellmont. 1958. Biochemical and pharmacological actions of Marsilid on the heart. J. Clin. Exptl. Psychopathol. **19:** Suppl. 1, 163.
10. Raab, W. 1953. Myocardial metabolism in pathogenesis and treatment of angina pectoris. Cardiologia. **22:** 291.
11. Loomer, H. P., J. C. Saunders & N. S. Kline. 1957. A clinical and pharmacodynamic evaluation of iproniazid as a psychic energizer. Psychiatric Research Repts. **8:** 129.
12. DeVerteuil, R. L. & H. E. Lehmann. 1958. Therapeutic trial of iproniazid (Marsilid) in depressed and apathetic patients. Can. Med. Assoc. J. **78:** 131.
13. Block, R. G., A. S. Dooneief, A. S. Blackberg & S. Spellman. 1954. The clinical effect of isoniazid and iproniazid in the treatment of pulmonary tuberculosis. Ann. Intern. Med. **40:** 881.
14. Bosworth, D. M., H. A. Wright, J. W. Fielding & H. J. Wilson. 1953. The use of iproniazid in the treatment of bone and joint tuberculosis. J. Bone and Joint Surg. **35A:** 577.

15. NUSSBAUM, H. E., W. LEFF, V. D. MATTIA, JR. & E. HILLMAN. 1957. The effects of iproniazid phosphate (Marsilid) on hypertension. Angiology. **8:** 198.
16. MASTER, A. M., S. DACK & H. L. JAFFE. 1938. Operation: a cause of coronary occlusion. J. Mt. Sinai Hosp. **4:** 980.
17. MASTER, A. M., H. L. JAFFE, L. E. FIELD & E. DONOSO. 1956. Acute coronary insufficiency: its differential diagnosis and treatment. Ann. Intern. Med. **45:** 561.
18. SCHERBEL, A. L. 1957. III. The effect of isoniazid and of iproniazid in patients with rheumatoid arthritis. Cleveland Clinic Quart. **24:** 90.

Discussion of the Paper

RICHARD J. BING (*Washington University Medical Service, St. Louis, Mo.*): Normal dogs were given iproniazid for 2 weeks to determine whether coronary blood flow changed significantly. Coronary blood flow was measured in each dog by the nitrous oxide desaturation method before starting the drug, and again after 2 weeks of its administration at 50 mg. twice daily. Hematocrit, blood pressure, and cardiac output were also measured. Dogs were excluded from the series if there were significant variations in any of the parameters from the control to the final experiment. No change in coronary blood flow, myocardial oxygen usage, or coronary sinus oxygen occurred (TABLE 1). Although these studies show clearly that iproniazid produces no change in coronary blood flow, the experiments will be continued to acquire a larger series. Other series of experiments now being undertaken deal with the influence of iproniazid on storage and production of catecholamines in heart muscle. Results of experiments undertaken in this laboratory have suggested that the cardiac efficiency diminishes immediately after interruption of the continuity of the heart with other organs and continues to diminish with time. Thus, the efficiency of the heart in the heart-lung preparation is greatly diminished. It is likely that this progressive loss of efficiency is the result of depletion of catecholamines. Iproniazid will be used to investigate this question.

TABLE 1
EFFECTS OF IPRONIAZID ON CORONARY BLOOD FLOW AND MYOCARDIAL OXYGEN USAGE

Coronary blood flow (cc./min./100 gm. L.V.)		Myocardial O_2 usage (cc./min./100 gm. L.V.)		Coronary sinus O_2 (vol. per cent)	
Control	After iproniazid	Control	After iproniazid	Control	After iproniazid
93	101	12.4	12.4	6.22	3.13
101	77	15.2	8.7	2.05	2.61
125	100	17.4	14.7	2.41	0.49
87	85	10.3	10.8	2.19	3.99
101	47	16.0	5.0	2.37	0.93
Av. 101	82	14.3	10.3	2.99	2.23

JOSEPH E. F. RISEMAN (*Beth Israel Hospital, Boston, Mass.*): The clinical results reported by Cesarman, Cossio, Schweizer, and Master on the use of iproniazid in patients with angina pectoris have been confirmed, in the main, by objective studies in a small group of patients at the Beth Israel Hospital.

In the latter studies, one third of the patients showed a moderate but significant increase in their ability to exercise, under standardized conditions, following 2 weeks of treatment with Marsilid (50 mg., t.i.d.). Another one third of the patients showed a slight but definite increase in exercise tolerance, while the remaining one third showed no response.

In general, the increase in exercise tolerance that followed the administration of Marsilid was less than that seen in the same patients following effective nitrites such as glycerol trinitrate or erythrol tetranitrate, given sublingually.

The period of administration of Marsilid was often accompanied by an improved sense of well-being. As a result, clinical improvement was more frequent and more striking than objective improvement.

This lack of close correlation of the results with Marsilid and those with nitroglycerin (as shown by our measurements and by the electrocardiographic studies of Cossio and Master) is important, because it suggests that the beneficial effects of iproniazid are due to a mechanism other than vasodilation. Hence, the drug might well afford a new approach to the treatment of angina pectoris, and the enhanced effect of nitroglycerin and the longer acting nitrites, as shown by Cossio, is not unexpected.

The untoward effects in our small series of patients who received the medication for only two weeks were infrequent and very mild. However, during the period of study, three cases of noncardiac patients with liver damage concurrent with iproniazid administration (one resulted in death) were reported from our hospital by Zetzel and Kaplan. This serious complication, although rare, contraindicates the use of this drug in the usual case of angina pectoris in which the immediate prognosis is good. Since iproniazid does not correct the underlying pathology in angina pectoris, the untoward effects weigh against its use in coronary artery disease. Fortunately, however, a group of Marsilid analogues is being investigated clinically. It can be expected that some of these will retain the beneficial effects of Marsilid while eliminating its toxic effects.

MILTON SHOSHKES (*Beth Israel Hospital, Newark, N. J.*): The initial experiences of Cossio[1, 2] and Cesarman[3, 4] with iproniazid in ischemic heart disease showed this drug to be of tremendous potential value. Reports (in this monograph) of further experiences with this medication reinforce the initial opinion that an excitingly new mode of symptomatic therapy for angina pectoris is available. However, two immense obstacles to wide acceptance of this drug remain: first the lack of an accurate double-blind type of experiment, such as that designed by Katz[5] and by Beecher,[6] tempers our enthusiastic acceptance of the drug for this indication, for there is the lingering thought that this may be still another new therapeutic of a long line of initially promising but eventually disappointing drugs; second, the enormously high rate of serious side reactions, which Master emphatically underscores, makes this drug unsafe for general distribution.

To deal with the first obstacle, we have just completed, at Beth Israel Hospital, a 6-month double-blind study with iproniazid. This study was arranged with 4 unknowns, 2 of which were 25-mg. iproniazid tablets, and the others identical placebos. The patients were presented with each tablet in random fashion at 1-month intervals, according to the following procedure: bottles containing placebo, iproniazid, iproniazid, and placebo were labeled *A*, *B*, *C*, and

D, respectively. The patient was started on any one of these, depending upon the period during which he joined the study. For example, if he began during the second week, he received medication in the order *B*, *C*, *D*, *A*. When the the code was broken, it was found that all but 5 of the patients had received iproniazid for 2 successive periods (*B* and *C*).

The daily dose of iproniazid was one 25-mg. tablet, b.i.d. Thus, theoretically, if the lower dose was successful, the rate of toxic reactions could be reduced. This trial was undertaken in spite of the previously reported lack o success with doses of less than 150 mg. per day.

Results

Effect on angina pectoris. The results (TABLE 1) were classified according to the method used by Blumgart *et al.*,[7] "excellent" being a 75 to 100 per cen reduction in frequency and severity of angina; "good," a 50 per cent reduction and "poor," a worsening or a 25 per cent (or less) reduction of the condition Upon the first administration of the placebo, results were 16 per cent excellen and 16 per cent good. With iproniazid, a marked improvement in respons appeared, with excellent results in 41 per cent and good in 23 per cent during the first month, and with excellent results in 62 per cent and good in 10 per cent during the second month—that is, a total of 64 and 72 per cent effectiveness, respectively. We found that responses were somewhat slower to appea than those noted by others, occurring 2 to 4 weeks after medication was begun The effects, in view of the fact that symptomatic benefit increases with time apparently were cumulative.

TABLE 1
SUMMARY OF SYMPTOMATIC EFFECT OF IPRONIAZID
UPON THE SYMPTOMS OF ANGINA PECTORIS

Response	Placebo		Iproniazid		Iproniazid		Placebo	
	No.	%	No.	%	No.	%	No.	%
Excellent	3	16	9	41	13	62	1	5
Good	3	16	5	23	2	10	5	25
Poor	13	68	8	36	6	28	14	70

Substitution of the placebo caused a marked and sudden falling off of improvement, with only 5 per cent of the patients claiming an excellent respons and 25 per cent a good response—a total of 30 per cent. The total of "excellent" and "good" responses during both placebo periods was similar, as wer the results with the 2 iproniazid trials. The evidence of cumulation is demonstrated in the increasing number of excellent responses after the second month as compared to the first. Furthermore, this favorable response was followe by a quick deflation on reinstitution of the placebo. This loss of therapeuti effect also was noted within 2 to 4 weeks.

Effect on the blood pressure. As shown in TABLE 2, with the first administration of the placebo, 11 per cent of the patients showed higher and 21 pe cent lower blood pressure. All changes were mild and fluctuating. Simila mild changes were found during the first month on iproniazid, with 14 per cent of the patients elevated and 23 per cent depressed. By the second month of administration of the test drug, however, 43 per cent showed a blood-pressure depression severe enough in 1 case to cause postural hypotension symptoms with mild syncope. These were relieved by discontinuing the drug for 2 weeks, after which it was safely reinstituted at a 25 mg./day level. Only 5 per cent showed an elevation at this time. A quick reversal appeared upon changing the drug to the placebo, and a rebound elevation was seen in 40 per cent, only 10 per cent becoming depressed. This, again, is evidence of a slow cumulative effect of the drug.

Effect on weight. Weight fluctuations were the least prominent of all observations (TABLE 3). On the first placebo trial, 32 per cent of the patients gained small amounts, and 21 per cent lost similar amounts. The results with the first iproniazid administration were essentially the same, with gain in weight in 23 per cent and loss in 18 per cent. By the second month of iproniazid medication, 38 per cent has gained slightly, while the percentage losing weight remained almost the same—that is, 19 per cent. The second substitution of the placebo produced a definite change, 50 per cent losing weight and 25 per cent gaining. This again would confirm a cumulative effect of iproniazid, as manifested by better appetite and nourishment, with a sudden downward rebound upon shifting to the inert material.

TABLE 2

SUMMARY OF EFFECT OF IPRONIAZID UPON BLOOD PRESSURE

Blood pressure	Placebo		Iproniazid		Iproniazid		Placebo	
	No.	%	No.	%	No.	%	No.	%
Higher	2	11	3	14	1	5	8	40
Unchanged	13	68	14	63	11	52	10	50
Lower	4	21	5	23	9	43	2	10

TABLE 3

SUMMARY OF EFFECT OF IPRONIAZID UPON WEIGHT

Weight	Placebo		Iproniazid		Iproniazid		Placebo	
	No.	%	No.	%	No.	%	No.	%
Higher	6	32	5	23	8	38	5	25
Unchanged	9	47	13	59	9	43	5	25
Lower	4	21	4	18	4	19	10	50

Effect on mood and feeling of well-being. The placebo elevated the mood of 47 per cent, while only 16 per cent felt worse (TABLE 4). These percentages remained almost unchanged during the first trial of iproniazid, 46 per cent of the patients feeling better and 18 per cent worse. During the second trial with the drug, the same number (48 per cent) claimed improvement, but only

4 per cent felt worse. Administration of the placebo, however, finally reduced the number of those feeling better to only 25 per cent, while 15 per cent again felt worse. Thus, the cumulative effect again manifested itself in the form o a general elevation of mood in nearly the entire group under treatment, only 1 subject feeling worse. Discontinuance of the drug was reflected in a definite diminution in the sense of well-being.

Effect on untoward reactions. Untoward reactions appeared with each tria (TABLE 5). These side effects were adjudged mild when the patient merely noted them to be present, without other complaint. When the patient was disturbed by the severity or nature of the side reaction, we judged it severe. In no case was it necessary to discontinue the study, and in the case of only one patient was interruption of the iproniazid trials indicated. Since the patient subsequently tolerated another iproniazid tablet at the same dose, it is doubtfu whether this was a true drug idiosyncracy or toxicity effect.

TABLE 4

SUMMARY OF EFFECT OF IPRONIAZID ON MOOD AND FEELING OF WELL-BEING

Mood	Placebo		Iproniazid		Iproniazid		Placebo	
	No.	%	No.	%	No.	%	No.	%
Improved	9	47	10	46	10	48	5	25
Unchanged	7	37	8	36	10	48	12	60
Worsened	3	16	4	18	1	4	3	15

TABLE 5

SUMMARY OF UNTOWARD REACTIONS TO PLACEBO AND IPRONIAZID

Degree of Reaction	Placebo		Iproniazid		Iproniazid		Placebo	
	No.	%	No.	%	No.	%	No.	%
Mild	1	6	5	23	8	38	3	15
Severe	1	6	1	4	3	14	1	5
Total	2	12	6	27	11	52	4	20

Discussion

Placebos, although pharmacologically inert, have been demonstrated to produce a wide variety of toxic reactions and, in general, can worsen the symptoms of as many as 20 per cent of an experimental population.[8] Similarly, in our first attempt with a placebo, we produced 1 mild and 1 severe reaction. When on the placebos, the patients complained of headaches, dizziness, itching nausea, weakness, tremors, and diarrhea. With iproniazid, they experienced fatigue, weakness, drowsiness, nausea, emesis, headache, vertigo, diarrhea palpitations, insomnia, euphoria, and, in 1 case, postural hypotension with syncope.

The first use of iproniazid, however, produced a definite increase in incidence of untoward reactions of mild degree in 23 per cent and reactions of severe degree in 4 per cent. The latter reaction of repeated nausea and vomiting forced us to discontinue the drug *B*, but the subject successfully took drug *C* in the same dose level; both *B* and *C* were iproniazid. By the second month, 38 per cent experienced mild, and 14 per cent severe side reactions. Of these reactions, postural hypotension compelled halving of the dose. These mild and relatively unimportant reactions in 52 per cent of the cases again demonstrate a cumulation of pharmacological effect of the drug. Return to the placebo resulted in a reduction in the incidence of side effects: that is, mild reactions in 15 per cent and severe in 5 per cent.

The demonstration of an increasing number of reactions with prolonged use of the drug emphasized the need to determine its effectiveness at the lower dose level of 25 mg./day. We have been able to reduce the dose still further to the 25 mg./day level in 9 of 11 patients of this group, mild and minor untoward side effects being experienced by only 2. This reduction to a minimal therapeutic dose, with concomitant reduction in toxic symptoms, greatly increases the potential usefulness of this drug. It should be begun at 50 mg./day in divided doses and reduced to 25 mg. (or even less) when the anticipated therapeutic effect is obtained. Levels higher than 50 mg./day should not be used in the ambulatory patient.

References

1. Cossio, P. 1957. The treatment of angina pectoris and of other forms of pain due to ischemia with iproniazid phosphate. Prensa méd. arg. **44:** 2679–2693.
2. Cossio, P. 1958. The treatment of angina pectoris and other muscular pain due to ischemia with iproniazid and isoniazid. Am. Heart J. **56**(1): 113–118.
3. Cesarman, T. 1957. "Serendipity" in angina pectoris: preliminary report on a therapeutic finding. Arch. inst. cardiol. Méx. **27:** 563–580.
4. Cesarman, T. 1958. Marsilid in the treatment of angina pectoris. J. Clin. Exptl. Psychopathol. and Quart. Rev. Psychiat. Neurol. **19**(2): Suppl. 1. 169–175.
5. Katz, L. N. 1956. The design of proper experiments to investigate clinical angina pectoris and the importance of knowing the determinants of coronary flow in considering therapy of angina pectoris. Ann. N. Y. Acad. Sci. **64**(4): 505–527.
6. Beecher, H. K. 1955. Appraisal of drugs intended to alter subjective responses, symptoms. Report to the Council. J. Am. Med. Assoc. **158:** 399–401.
7. Blumgart, H. L., A. S. Freedberg & G. S. Kurland. 1955. Treatment of incapacitated euthyroid cardiac patients with radioactive iodine. J. Am. Med. Assoc. **157:** 1.
8. Wolf, G. & R. A. Pinsky. 1954. Effects of placebo administration and occurrence of toxic reactions. J. Am. Med. Assoc. **155:** 339–341.

J. L. Rivier (*Clinique Médical Universitaire, Lausanne*) and P. W. Duchosal (*Centre de Cardiologie de l'Université, Geneva, Switzerland*): We have been using iproniazid (Marsilid) for the treatment of angina pectoris since 1957. Our test series comprises 75 cases (52 in Lausanne, 23 in Geneva), observed for periods of from 2 to 11 months. The ages of these patients ranged from 35 to 80 years, with an average of 59 years.

As shown in table 1, the incidence of success was 75 per cent; 12 patients (16 per cent) derived no benefit; and 7 patients (9 per cent) expired during treatment. Table 2 shows the results of Marsilid therapy in the latter patients before their death.

The results presented in table 1 correspond more or less to those presented elsewhere in the monograph. The side effects observed initially prompted us to prescribe gradually increasing doses of Marsilid. Thus, treatment was instituted with a daily dose of 50 mg., which was increased by 25 mg. every third day until an effective dose level was reached or disturbing side reactions occurred. In many instances a daily dose of 75 or 100 mg. produced gratifying results. By increasing doses gradually the incidence of side effects could be reduced. In some cases unpleasant symptoms occurred after 5 to 7 days o treatment, but disappeared promptly on discontinuation of iproniazid; when the drug was reinstated it was tolerated well. In other patients side effects persisted for some time despite interruption of therapy and, in these cases, the interval without medication was somewhat longer. When Marsilid was reinstated the patients were advised to discontinue the drug as soon as side effects reappeared. For maintenance therapy we usually gave Marsilid for 4 or 5 days of the week or every other week.

We believe that iproniazid can be prescribed in this way for outpatients without any undue hazards. The patient must be warned, of course, that side effects, especially vertigo, may occur. In our opinion, however, the latter i not always due to postural hypotension. Seven of our patients complained o dizziness, but in only three of these was the vertigo definitely attributable t orthostatic hypotension. In some patients with persistent arterial hypertension small doses of reserpine were administered in addition to Marsilid. Naturally, in severe cases with frequent anginal attacks, violent and frequen crises, or marked electrocardiographic modifications, we also gave syntheti anticoagulants. Each patient received nitroglycerin as required.

TABLE 1
RESULTS OF TREATMENT WITH IPRONIAZID

Response	No. of patients	Percentages of total
Excellent	37	49.5
Good	19	25.5
None	12	16
Death	7	9

TABLE 2
RESPONSES OF PATIENTS WHO DIED DURING IPRONIAZID TREATMENT

Response	No. of patients
Excellent	3
Good	1
None	3

In all probability, the 7 deaths that occurred were due to myocardial infarctions. One woman who was 77 years of age and had suffered frequen anginal attacks for many years died on the third day of treatment with iproniazid, after having taken 75 mg. on the first day and 100 mg. each on th second and third days without the slightest complication.

A 62-year-old male whose anginal attacks were treated by rest, anticoagulants, vasodilators, and Marsilid died during the night. He had been receiving iproniazid for 2 weeks without side effects, but also without improvement

A 53-year-old man who had suffered a myocardial infarction in 1955 had a coronary occlusion at 5 o'clock in the morning after having been on intermittent Marsilid therapy for 6 weeks with good results.

Another 60-year-old male had a myocardial infarction in spite of rest, anticoagulants, vasodilators, and iproniazid (given for 8 days with no effect on the pain). He had been confined to bed throughout this period.

The fifth case was an 80-year-old man who had had an infarction 6 years previously and a cerebral episode 1 year before initiation of iproniazid treatment. A daily Marsilid dose of 100 mg., 5 days a week, completely relieved his anginal attacks for 3 months. He died at night from pulmonary edema that probably was due to a myocardial infarction.

The sixth patient was a 60-year-old male with hypertension, who had been suffering from angina pectoris for 8 years and was taking doses of 100 mg. of Marsilid intermittently for several months, with complete relief of pain. He never felt dizziness nor did he complain of any other unpleasant sensations; the cause of his sudden death during the day remains unexplained.

The last case is that of a 67-year-old man who was relieved of his pain after having received a daily Marsilid dose of 75 mg. for 8 days and who died suddenly on the eighteenth day of the treatment.

The question must be raised whether Marsilid is implicated in these fatalities. If it is, the mechanism cannot be solely that of drug-induced orthostatic hypotension and its sequelae, since three of the patients with a fatal outcome had been in bed for several hours and one had been confined to bed for eight days at the time of death. In these instances some other toxic effects on the myocardium would necessarily have been involved. However, the high death rate in all categories of coronary patients is well known and it is, therefore, altogether possible that these deaths were not attributable to the medication. Only prolonged, carefully controlled studies of large comparative series will answer this question. Whatever the cause of death, it is imperative that dosage be carefully adjusted and that the patients remain under close supervision during the entire treatment.

In this connection, the following observations in 7 emergency cases of myocardial infarcts admitted to the Clinique Médical Universitaire may be of interest. These patients who still have pain were given, in addition to the usual treatment with anticoagulants, analeptics, sedatives, and oxygen, 150 mg. Marsilid daily for periods of 10 to 30 days, depending on circumstances. In these instances iproniazid did not relieve the pain, nor did it alter the course of the disease but, at the same time, the drug had no untoward effects and none of the patients died.

It is noteworthy that, in angina of effort, Marsilid not only relieves the pain, but also increases the physical capacity of the patient. We have studied several such cases whose serial electrocardiograms revealed no changes typical of myocardial distress, so often seen during an attack in this type of patient. It is well known that a supplementary physical effort at this precise moment involves the patient in grave danger. If iproniazid exerted only an analgesic effect, our patients with angina of effort would have incurred a whole series of accidents, since they automatically increase their activity once the attacks disappear or diminish. In fact, 22 of our patients suffering from angina of effort have been able to increase their physical activity considerably. Only 1 died, an 80-year-old man who had had a myocardial infarction several years before and a slight stroke 13 months earlier. Thus, iproniazid cannot have merely an analgesic effect; there must be a direct action, at the level of the myocardium, on the complex phenomena resulting in the anginal attack in the coronary patient.

Whatever the mechanism of action of iproniazid, the fact remains for us clinicians that the results of treatment with this compound in angina pectoris are encouraging and that they are fundamentally different from those known to us from any other modality of treatment.

SIGNIFICANCE OF MONOAMINE OXIDASE INHIBITION FOR THE PHARMACOLOGICAL AND CLINICAL EFFECTS OF HYDRAZINE DERIVATIVES

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Monoamine oxidase (MAO) inhibitors of the hydrazine type have a variety of pharmacological and clinical effects. The question arises whether these actions are the consequence of MAO inhibition or whether MAO inhibition is a mere coincidental alteration, unrelated to the observed pharmacological effects. I shall summarize some findings that may elucidate the possible role of MAO inhibition as a basic mechanism for the action of hydrazides, mainly in the brain and heart.

The Effects of Hydrazides on Monoamine Metabolism

Endogenous monoamines. After administration of MAO inhibitors of the hydrazide type to animals,[1-5] an increase of monoamines [5-hydroxytryptamine (5-HT) and catecholamines (CA)] occurs in various organs. In humans, an increase of 5-HT was observed in blood platelets after repeated administration of iproniazid (Marsilid*).[5, 6] This monoamine rise lasts for several days after discontinuance of the drug, and is less marked or absent with hydrazides that have little or no inhibitory effect on MAO. The increase of monoamines is probably due to their accumulation in the tissues, because their breakdown is prevented by the action of MAO inhibitors.

MAO inhibitors, furthermore, counteract the changes in monoamine metabolism induced by certain *Rauwolfia* alkaloids and benzoquinolizines (BQ). The latter drugs cause a diminution of the monoamine content in the tissues, probably due to liberation and consecutive breakdown of the amines.[7-9] By pretreatment with MAO inhibitors, the reserpine- and BQ-induced decrease of the monoamines is inhibited.[2, 3, 10] The mechanism of this antagonism is not yet clear. It may be that the amines liberated from a bound state by reserpine or BQs are no longer broken down because of MAO inhibition. This would lead to an increase of "free" monoamines in the tissues. Another explanation may be that MAO inhibitors counteract the reserpine- and BQ-induced liberation of monoamines from the cells. Evidence for the latter explanation is provided by histochemical experiments with the chromaffin system in the intestine and the adrenal medulla.[11, 12]

Exogenous monoamines. MAO inhibitors enhance the effects of 5-hydroxytryptophan (5-HTPN) and dihydroxyphenylalanine (DOPA) on the monoamine content of the brain. When administered after pretreatment with Marsilid, 5-HTPN and DOPA cause a more marked elevation of 5-HT and CA, respectively.[1, 3] Both 5-HTPN and DOPA can pass the blood-brain barrier and are decarboxylated in the brain to the corresponding amines. As MAO is inactivated by Marsilid, the degradation of these amines is at least partly inhibited.

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* Hoffmann-La Roche.

Relation Between Effects of Hydrazides on Monoamine Metabolism and The Pharmacological Action in Animals

Three principal types of pharmacological effects of hydrazides are known t have a possible causative relation to MAO inhibition. These are enhancemer of the effect of certain monoamines and their precursors, antagonism agains reserpine and BQ, and anticonvulsive effect.

Enhancement of the effect of monoamines and precursors. When admini tered after pretreatment with Marsilid and other MAO inhibitors, monoa mines and precursors cause a more marked increase of body temperatur (TABLE 1).[13, 24, 25] Furthermore, the excitatory as well as the other pharmaco logical effects of 5-HTPN and DOPA are markedly enhanced by pretreatmer of the subject with different MAO inhibitors. An increase of the effects 5-HTPN and DOPA can still be seen several days after a single injection the MAO inhibitors. This is not the case for hydrazides without inhibitor action on MAO. Thus, there is evidence that the major cause for enhanc ment of the central effects of monoamines and precursors is MAO inhibition

TABLE 1

CHANGE IN BODY TEMPERATURE (°C.) IN RABBITS*

Drug		Pretreatment		
	Dose (mg./kg. I.V.)	None I	Marsilid (ipron-iazid) II	Rimifon (iso niazid) III
5-Hydroxytryptophan	10	+0.33	+1.82	
Dihydroxyphenylalanine	10	+0.68	+1.40	
5-Hydroxytryptamine	25	+0.34	+1.83	−0.14
Adrenaline	0.25 s.c.	−0.07	+0.77	
Tyramine	40	+0.57	+2.87	

* The hydrazides were administered I.P. in doses equimolecular to 100 mg./kg. Marsi 16 hours before I.V. injection of the amines and precursors. The figures represent me values of 3 to 4 experiments each. The differences between the values in columns I and are significant ($p < 0.01$); those in columns I and III are not ($p > 0.05$).

Reserpine antagonism. Some central pharmacological effects of reserpi and BQ are antagonized or reversed by pretreatment of subjects with vario MAO inhibitors. Thus, the sedative action of reserpine and BQ, as well as t prolongation of ethanol and barbiturate narcosis, are diminished in mice. rabbits, after administration of MAO inhibitors, reserpine, and BQs no long cause sedation, hypothermia, and miosis, but produce excitation, hypertherm mydriasis, and piloerection.[10, 14-16] These effects of the hydrazides seem to pa allel their inhibitory action on MAO, and are not produced by weak MAO i hibitors.[17] This counteraction is not the consequence of any direct stimula ing effect of these compounds on the central nervous system (CNS); this evident from the fact that they have no immediate influence on the acti of reserpine and BQ if they are administered after the latter drugs, at a ti when reserpine and BQ already have depleted the monoamines stored in t brain.[10, 16] Further evidence that interference with monoamine metaboli plays a role in the reversal of reserpine action is given by experiments wi chlorpromazine. This drug, like reserpine, causes hypothermia. However, it produces no release of monoamines in the brain. Pretreatment of the subject with MAO inhibitors does not cause a reversal of the chlorpromazine effect on body temperature; on the contrary, the hypothermic effect of the drug is enhanced (TABLE 2).[13]

TABLE 2

CHANGE IN BODY TEMPERATURE (°C.) IN RABBITS*

Drug		Pretreatment		
	Dose (mg./kg., I.V.)	None I	Marsilid (iproniazid) II	Rimifon (isoniazid) III
Reserpine	1.0	−0.98	+3.22	−0.89
Chlorpromazine	5.0	−0.61	−1.03	

* Administration of the hydrazides was as described in TABLE 1. The figures represent mean values for 3 to 4 experiments each. The difference between the reserpine values in column I and II are significant ($p < 0.01$); those in columns I and III are not ($p > 0.05$). The chlorpromazine values are not highly significant ($p \sim 0.05$).

TABLE 3

EFFECT OF MAO INHIBITORS ON BLOOD PRESSURE IN ANIMALS AND MAN*

Compound	MAO inhibition in brain†	Effect on blood pressure	
		Animals	Humans
N^2-isopropylhydrazide of isonicotinic acid	1.0	−	Orthostatic hypotension
D,L-Serine	2.5	(−)	Orthostatic hypotension
p-Chlorobenzoic acid	1.7	0	Orthostatic hypotension
N-Acetyl-D,L-methionine	1.6	0	Orthostatic hypotension
L-Glutamic acid	2.9	+	Orthostatic hypotension
L-Alanine	3.5	++	Orthostatic hypotension
Isonicotinic acid hydrazide	<0.1	0	None

* The action of blood pressure in cats was determined immediately after I.V. injection; the blood pressure effect in humans after prolonged administration of the drugs. Hydrazide doses are equimolecular to 100 mg./kg. Marsilid (MAO inhibition) and 50 mg./kg. Marsilid (effect on blood pressure in animals).

† Figures indicate the following quotient: ED_{100} Marsilid/ED_{100} drug indicated in which ED_{100} represents the dose of the drug that produces a 5-HT increase of 100 per cent in the brains of rats 16 hours after I.P. administration (spectrophotofluorometric method).

The possible mechanism of this antagonism between MAO inhibitors and reserpine or BQ has been mentioned above. It might be explained by the fact that MAO inhibitors counteract the reserpine- and BQ-induced release of monoamines. The reversal of reserpine and BQ action in rabbits is possibly due to an increased amount of "free" monoamines in the brain, causing central stimulation.

Anticonvulsive effect. It has been shown that MAO inhibitors have anticonvulsive effects in animals. This is true of MAO inhibitors of various chemical types (Marsilid, phenylisopropylhydrazine, harmaline), and the effect seems to parallel the increased monoamine levels produced in the brain b these drugs.[18] On the other hand, reserpine, which causes a decrease of mono amines in the brain, facilitates convulsions. Furthermore, the reserpine-induced facilitation of convulsions can be counteracted by Marsilid.[18,19] Thus it seems probably that there exists some causal connection between anticonvulsive effect and MAO inhibition. The mechanism of action that leads t this anticonvulsive activity is, however, not yet known.

In addition to the effects mentioned above, some pharmacological action of hydrazides are probably not due to MAO inhibition. Thus, various hydrazides, when injected intravenously, have an immediate effect on blood pressure.[20] This action does not parallel that on monoamine metabolism. Furthermore, the effects of hydrazides, caused by their interference with vitami B_6 (inhibition of B_6-containing enzyme systems such as transaminase and th codehydrases), are probably not related to MAO inhibition. Thus, the enhancement of urinary B_6 excretion by certain hydrazides is not related to thei potency as MAO inhibitors.[21]

Relation Between Effects of Hydrazides on Monoamine Metabolism and Their Clinical Actions

The two principal therapeutic effects of hydrazides are beneficial activit in certain forms of mental depression and alleviation of angina pectoris. I cannot yet be decided whether MAO inhibition is the most important mechanism for these actions. The following findings indicate some causal relationship:

(1) With the small doses of hydrazides used in humans, MAO inhibitio becomes significant only after several days of repeated administration. Th is explained by the long-lasting and therefore cumulative effects of these drug on MAO. The onset of clinical action of the hydrazides, too, occurs generall only after several days.

(2) After discontinuance of the hydrazide medication it is at least severa days until the elevated monoamine content in the organs of experimental animals returns to normal. The clinical effects, also, outlast by several days th discontinuance of the drugs.

(3) The clinical effectiveness of hydrazides roughly parallels their activit on monoamine metabolism (TABLES 4 and 5).

It is of interest that isopropylhydrazide of L-glutamic acid seems to have biphasic effect in humans. During the first days of medication it seems t produce a depressant effect on the CNS which, after a short period of treatment, changes into the typical antidepressant action of Marsilid. It is no known whether this initial depressant effect is due to MAO inhibition or to direct action of the compound on the CNS.[26]

Experience with additional hydrazine derivatives is needed to determi whether the clinical effects of these drugs are always associated with an alter tion of monoamine metabolism, and whether these changes are responsib for some of the pharmacological action in humans.

The two major clinical side effects of monoamine oxidase inhibitors consi in hypotension and liver toxicity.

The hypotensive effect seems to be of the postural type. It is not yet cle whether this type of action will be useful in therapy. Until now, every MAO inhibitor tested in humans showed hypotensive activity (TABLE 3). However, it is not yet known whether this action depends on the inhibitory effect of the drugs on MAO. Existing theories on the mechanism of this hypotensive effect have not yet been definitely supported.

TABLE 4

CORRELATION BETWEEN MAO INHIBITION IN THE BRAIN AND CLINICAL ACTIVITY OF MAO INHIBITORS*

Compound	MAO inhibition in brain†	Clinical activity (mental depression)
N^2-isopropylhydrazide of isonicotinic acid.	1.0	+
p-Chlorobenzoic acid	1.7	+
N-acetyl-D,L-methionine	1.6	+
D,L-Serine	2.5	++
L-Glutamic acid	2.9	− +
N^2-benzylhydrazide of picolinic acid	4.9	++
Meth. isoxaz. carbox. acid	5.9	++
Isonicotinic acid hydrazide	<0.1	(+?)

* The data on human subjects were obtained through the courtesy of various clinical collaborators.

† For explanation of the figures, see TABLE 3.

TABLE 5

EFFECT OF MAO INHIBITORS ON CATECHOLAMINE CONTENT OF THE HEART AND ON ANGINA*

Compound	CA increase in the heart	Clinical effect in angina
N^2-isopropylhydrazide of isonicotinic acid	100 ± 15	+
N-acetyl-D,L-methionine	60 ± 11†	(+)
p-Chlorobenzoic acid	80 ± 25	+
L-Glutamic acid	75 ± 12	+
L-Alanine	110 ± 20	+
Isonicotinic acid hydrazide	30 ± 12†	(+?)

* The catecholamine content of the myocardium of guinea pigs was measured 16 hours after I.P. injection of the drugs in doses equimolecular to 100 mg./kg. Marsilid (spectrophotofluorometric method). Information on the effects of the drugs in angina pectoris was obtained through the courtesy of various clinical collaborators.

† Significant difference as compared to the values with N^2-isopropylhydrazide of isonicotinic acid (Marsilid).

The pathogenesis of the liver toxicity of hydrazides in humans is not known. There is the possibility that the drug activates a latent hepatitis virus or that it worsens the course of manifest viral hepatitis. It is not known, however, whether the liver damage in humans is related to the effect of the drugs on MAO. Further clinical experience with other MAO inhibitors is needed to show whether toxic effects on the liver are inherent in this type of compound. In addition, studies on drug metabolism seem to be fruitful in the elucidation of the difficult problem of liver toxicity.[22]

The finding mentioned above represent evidence that MAO inhibition somehow plays a role in certain pharmacological actions of MAO inhibitors of the hydrazide type. The mechanism whereby MAO inhibition and a disturbed monoamine metabolism produce these effects in humans and in animals, however, is not yet known. The possibility must be considered that accumulation of monoamines in the tissues or inhibition of the release of monoamines from tissue structures is directly responsible for some effects of the drugs. Furthermore, it is conceivable that accumulation of metabolic breakdown products of monoamines causes some of these effects. Thus, it has been shown that, under the influence of Marsilid, 3-*O*-methyl-norepinephrine, a metabolic product of norepinephrine, accumulates in the brain.[23] It is not known whether this metabolite, after penetration of the blood-brain barrier, exerts great pharmacological activity. Furthermore, it is possible that other, thus far unknown, metabolites also accumulate and play a role in the clinical effects of the MAO inhibitors.

One possible consequence of such changes in monoamine metabolism may be an alteration of nervous transmission, leading to some of the central and other pharmacological effects. Such considerations, still theoretical, remain to be proved but, at present, they furnish a valuable working hypothesis.

Summary

I have summarized those experimental and clinical findings that may serve to elucidate the role of MAO inhibition in the pharmacological and clinica actions of hydrazides. There is good evidence to show that, for certain centra actions of hydrazides in animals (potentiation of monoamines and precursors antagonism to reserpine, antiepileptic effect), MAO inhibition plays an important role. For other pharmacological effects, such as B_6 excretion and acute effect on blood pressure, this is not the case. Furthermore, within a series of hydrazide derivatives there is a rather good correlation between the influence on monoamine metabolism in animals and its clinical effects in humans (mental depression, angina pectoris, blood pressure regulation). Further clinical experience with additional hydrazine derivatives is needed to establish whether interference with monoamine metabolism is essential for the effects o hydrazides in man.

References

1a. Udenfriend, S., H. Weissbach & D. F. Bogdanski. 1957. Ann. N. Y. Acad. Sci 66(3): 602.
1b. Udenfriend, S., H. Weissbach & D. F. Bogdanski. 1957. J. Pharmacol. Exptl Therap. **120:** 255.
2a. Pletscher, A. 1956. Experientia (Basel). **12:** 479.
2b. Pletscher, A. 1956. Helv. Physiol. et Pharmacol. Acta. **14:** C76.
3. Pletscher, A. 1957. Schweiz. med. Wochschr. **87:** 1532.
4a. Spector, S., D. Prockop, P. A. Shore & B. B. Brodie. 1958. J. Pharmacol. Exptl Therap. **122:** 71A.
4b. Spector, S., D. Prockop, P. A. Shore & B. B. Brodie. 1958. Science. **127:** 704.
5. Pletscher, A. & A. Bernstein. 1958. Nature. **181:** 1133.
6. Shore, P. A., L. Gillespie, S. Spector & D. Prockop. 1958. Naturwiss. **45:** 340.
7. Pletscher, A., P. A. Shore & B. B. Brodie. 1956. J. Pharmacol. Exptl. Therap **116:** 84.
8. Pletscher, A. 1957. Science. **126:** 507.

9. Pletscher, A., H. Besendorf & H. P. Bächtold. 1958. Naunyn-Schmiedeberg's Arch. exptl. Pathol. u. Pharmakol. **323:** 499.
10. Shore, P. A. & B. B. Brodie. 1957. Proc. Soc. Exptl. Biol. Med. **94:** 433.
11. Zbinden, G., A. Pletscher & A. Studer. 1957. Klin. Wochschr. **35:** 565.
12. Zbinden, G. & A. Studer. 1958. Experientia (Basel). **14:** 201.
13. Bächtold, H. & A. Pletscher. 1957. Experientia (Basel). **13:** 163.
14. Chessin, M., B. Dubnick, E. R. Kramer & C. C. Scott. 1956. Federation Proc. **15:** 409.
15. Brodie, B. B., A. Pletscher & P. A. Shore. 1956. J. Pharmacol. Exptl. Therap. **116:** 9.
16. Besendorf, H. & A. Pletscher. 1956. Helv. Physiol. et Pharmacol. Acta. **14:** 383.
17. Bächtold, H. P. Personal communication.
18. Prockop, D., P. A. Shore & B. B. Brodie. 1958. Ann. N. Y. Acad. Sci. **80**(3): 643.
19. Hertting, G. 1958. Wien. klin. Wochschr. **70:** 90.
20. Pellmont, B. Personal communication.
21. Weber, F. & O. Wiss. Personal communication.
22. Koechlin, B. & V. Iliev. Ann. N. Y. Acad. Sci. **80**(3): 864.
23. Axelrod, J. 1958. Science. **127:** 754.
24. Horita, A. 1958. J. Pharmacol. Exptl. Therap. **122:** 176.
25. Horita, A. & H. J. Gogerty. 1958. J. Pharmacol. Exptl. Therap. **122:** 195.
26. Voelkel, A. Personal communication.

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